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(FIZIOLOGIIA RASTENII)

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THE EFFECT OF 2,4-D ON NITROGEN METABOLISM IN OAT AND BEAN PLANTS

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It has been established that under the influence of 2,4-D (2,4-dichlorophenoxyacetic acid) a large number of changes take place in the metabolism of dicotyledons; there is observed a sharp supression of photosynthesis [1-5], a very considerable change in the rate and nature of respiration [4, 6-12], a decrease in nitrogen uptake [13], and in phosphorus uptake [14-16], a great change in the usual course of transformation of carbohydrate [7, 17-24], and of nitrogenous substance [7, 10, 13, 18, 19, 22, 25-28], a decrease in formation of such functionally important substances as nucleoproteins and phosphatides [29-31], a significant change in the colloidal-chemical properties of protoplasm [32-35], and other aspects of life activity. The present data allow one to conclude that a sharp decrease in synthetic processes and an increase in anabolic processes, a strong inhibition of metabolism and irreversible protoplasmic injury form the basis of the herbicidal action of 2,4-D and other substances [36-41].

Insofar as the effect of 2,4-D on the metabolism of cereals and the causes for their high resistance to 2,4-D are concerned, there exist only individual papers [24, 42-44]. The internal causes of the dissimilar sensitivity of dicotyledonous plants towards substances of the 2,4-D type have been little studied. Yet, the clarification of this question has not only theoretical interest but is also of great significance for the practical application of this compound and for further work in the direction of synthesis of more effective herbicides.

In the present paper, we describe our investigations concerning the action of 2,4-D on the nitrogen metabolism of oat and bean plants, which differ strongly from each other in their sensitivity to this compound.

METHODS

The bean variety "Latvia" and the oat variety "Moskovskii A-315" were used as experimental plants. Plants were grown in culture on Knop's nutrient solution, with addition of the microelements Cu, Zn, Mn, B, and Fe. The herbicide 2,4-D was applied as the sodium salt. Treatment of plants was carried out in the period of their greatest sensitivity to the herbicide, namely – of beans, at the time of appearance of the second true leaf, and of oats, at the time of appearance of the third, fourth, and fifth leaves. The treatment consisted in spraying the plants (with an atomizer) with a 0.1% solution of 2,4-D, at the rate of 5 ml of solution per container of plants (the diameter of the containers was 25 cm). To prevent contamination of the culture substrate by the solution, the surface of the sand in the container was covered with cotton prior to treatment.

After spraying, we used the method of vacuum infiltration of solution into the intact plant, grown in a sand culture which we have developed. Prior to our experiments, infiltration had been carried out only on intact plants that were grown under the conditions of water culture [45].

Plants that were to be used for vacuum infiltration were planted in trays of Mitcherlikh vessels. Quartz sand, with Knop's nutrient mixture added, served as a substrate. Seedlings were thinned out as soon as they appeared, so that 12 oat plants or 8 bean plants were left in the containers. After that, leatherette circles, with round openings cut out for the plants, were placed on the surface of the sand.

These circles were left in the vessels until the end of the experiment, and prevented spilling of the sand when vessels were inverted, which is necessary for the infiltration of plants. Infiltration was carried out in a large vacuum desiccator (volume 12 liters), while the 2,4-D solution was used in such amounts that almost all

TABLE 1

Effect of 2,4-D on Content of Nitrogenous Substances in Whole Bean Plants After Spraying With Herbicide

Time after treatment at which samples were taken	Experimental treatments	Total nitrogen		Protein nitrogen		Nonprotein nitrogen	
		in mg per 100 plants	in %	in mg per 100 plants	in %	in mg per 100 plants	in % of total nitrogen
Second day	Control	1828	100	1446	79.4	381	20.6
	2,4-D	1785	100	1350	75.5	435	24.5
Fourth day	Control	1946	100	1496	77.0	450	23.0
	2,4-D	1759	100	1322	75.3	437	24.6
Eighth day	Control	2859	100	2240	78.6	619	21.4
	2,4-D	1857	100	1316	70.8	541	29.2

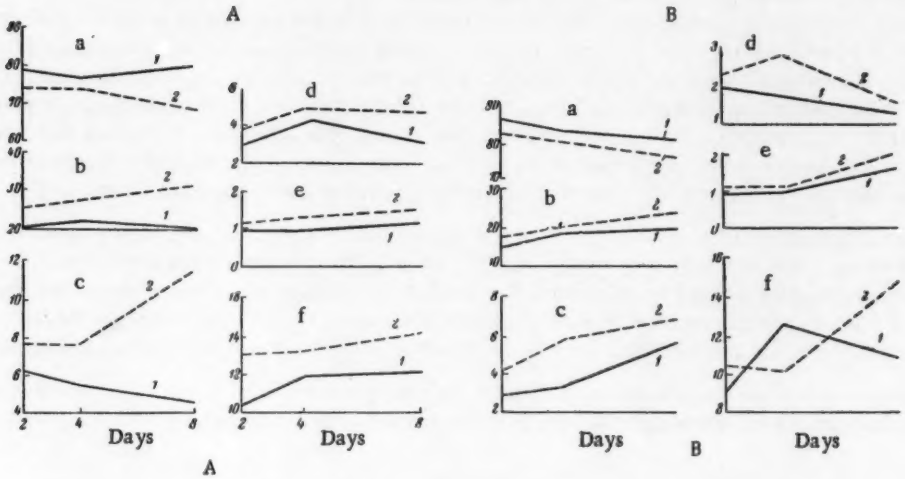

Fig. 1. Content of various forms of nitrogenous compounds (in % of total N) in the whole plants of beans (A) and oats (B) after infiltration with 2,4-D solution.
a) Protein N; b) nonprotein N; c) amino acid N; d) amide N; e) ammoniacal N; f) residual N; 1) control; 2) 2,4-D treated.

the aerial parts of the plants were immersed in it; 1 to 1.5 cm reserve space was left between the surface of the solution and the top of the container in the desiccator. The container of plants was placed on an iron stand, covered with a thin layer of paraffin, inside the desiccator. The necessary vacuum was obtained inside the desiccator with the aid of an electric oil pump. The pump was left running until evolution of air bubbles from the plants ceased. Depending on the object and on the phase of its development, the evacuation of air continued from 5 to 10 minutes; then the pump was stopped, and air was gradually let into the desiccator in such a way that the pressure would come to equilibrium within 2 minutes. To prevent contamination of the sand by 2,4-D solution, the aerial part of the plants was thoroughly washed after infiltration. This was done by repeated immersion of the aerial parts of the plants, first into tap water and then into distilled water. As controls, plants infiltrated with distilled water, were used. For faster removal of water from the intercellular spaces, all infiltrated plants were placed in sunlight.

Samples of plant material for analysis were taken on the second, fourth, eighth, and sometimes the sixteenth day after treatment. At each of these times plants from two control and two experimental containers were taken for analysis, and plants were then separated into leaves, stems, and roots, which were analyzed separately.

TABLE 2

Changes in Content of Various Forms of Nitrogen in mg N in Separate Organs of Beans After Spraying Plants With 2,4-D Solution

Time of sampling	Experimental treatments	Leaves				Stems				Roots			
		Total N		Protein-N		Nonprotein N		Total N		Protein N		Nonprotein N	
		per 100 plants	per g dry wt.	per 100 plants	per g dry wt.	per 100 plants	per g dry wt.	per 100 plants	per g dry wt.	per 100 plants	per g dry wt.	per 100 plants	per g dry wt.
Second day	Control	930	45.50	794	38.75	136	6.75	550	20.62	372	14.17	178	6.45
	2,4-D	605	46.40	590	38.48	120	7.92	690	24.40	462	16.35	228	8.05
Fourth day	Control	1010	38.40	833	31.60	177	6.70	493	14.65	316	9.39	177	5.26
	2,4-D	605	41.80	490	33.85	115	7.95	750	26.60	516	18.30	234	8.30
Eighth day	Control	1610	42.10	1328	34.70	282	7.40	635	15.10	417	9.90	218	5.20
	2,4-D	592	40.04	465	31.38	127	8.66	825	29.20	501	17.70	324	11.50

Time of sampling	Experimental treatments	Roots			
		Total N		Protein N	Nonprotein N
		per 100 plants	per g dry wt.	per g dry wt.	per g dry wt.
Second day	Control	348	23.40	18.56	4.84
	2,4-D	385	24.70	19.09	5.61
Fourth day	Control	443	23.20	18.16	5.04
	2,4-D	404	24.50	19.14	5.36
Eighth day	Control	614	23.40	18.85	4.55
	2,4-D	440	29.70	23.60	6.10

Time of sampling	Expt. treatments	Protein N per 100 plants	Nonprotein N per 100 plants
Second day	Control	276	72
	2,4-D	298	87
Fourth day	Control	347	96
	2,4-D	316	88
Eighth day	Control	495	119
	2,4-D	350	90

Analysis of the content of various forms of nitrogen was carried out both on fresh and on dry material. In the later case, the plants were previously fixed at 105° for 15 minutes, and then were dried at 60° to the air dry state. Total nitrogen was measured by the micro-Kjeldahl method [46]. The nonprotein nitrogen was determined (in the filtrates after protein precipitation) according to Barnshtein (Barnstein) [46]. Protein nitrogen was calculated from the difference between total and nonprotein nitrogen. Amino acid nitrogen was determined by the Van Slyke method [46]. In addition, the synthesizing activity of protease was measured by the Kursanov method [48]. The hydrolyzing activity of protease was determined on cut plants which were placed in a dark chamber, at a temperature of 30°, for 40 hours.

At first, we shall consider the data which characterize the changes in nitrogen metabolism in plants after the spraying treatment (the data of one of the experiments are presented in Tables 1, 2, and 3).

From Table 1, it can be seen that nitrogen uptake is sharply decreased in plants treated with 2,4-D; in treated plants, the absolute nitrogen content is actually unchanged throughout the entire experiment, whereas in the controls, it continually increases. Toward the eighth day of the treatment the nonprotein nitrogen portion is somewhat increased in treated plants, while the content of protein substance decreases insignificantly.

Our observations, demonstrating the inhibiting action of 2,4-D on nitrogen uptake, are in complete agreement with the available data [13, 16, 28, 39] on the inhibiting effect of the herbicide on the absorption by the plant of the elements of mineral nutrition. Nance [50] connects this phenomenon with a direct inhibiting action of 2,4-D on the absorption capacity of roots. But the possibility is not excluded that the observed retardation of nitrogen introduction into the plant is also directly connected with a direct action of the herbicide on the root system. The experiments in which it has been shown that herbicide introduced into the leaves is rapidly found in the root system [51] also speak in favor of this possibility.

TABLE 3

Effect of 2,4-D on Distribution of Nitrogenous Substances Among the Organs of Bean Plants
(in % of total content of nitrogenous substance in plant)

Time of sampling	Expt. treatments	The whole plant	Leaves	Stems	Roots
Second day	Control	100	50.8	30.0	19.0
	2,4-D	100	39.5	38.6	21.5
Fourth day	Control	100	51.9	25.3	22.7
	2,4-D	100	34.3	42.6	23.0
Eighth day	Control	100	56.3	22.2	21.4
	2,4-D	100	31.3	44.4	23.7

In addition, taking into account the deep interconnection of the functional activity of the roots and the aerial systems [52-54], one may consider that the weakening of the process of nitrogen absorption is determined not only by the direct action of 2,4-D on roots, but also indirectly, through the breakdown of synthetic processes in plants.

In order to have a concept of the action if 2,4-D on the nitrogen metabolism in different organs of the plants, we have carried out the necessary analysis, the results of which are given in Table 2.

As can be seen in Table 2, the absolute content of protein nitrogen is greatly decreased in the leaves of treated plants, but the content of nonprotein nitrogen is almost not increased at all, which indicates a transfer of nitrogenous substances from leaves into other plant organs. A certain increase in the relative content of protein and nonprotein nitrogen on the second and fourth day from the beginning of the experiment is evidently connected with the intensive expenditure of non-nitrogenous substances for respiration, which is noticeably increased in the treated plants as a rule [6-10].

In the stem a reverse process takes place, namely: the relative, as well as the absolute, content of protein and nonprotein nitrogenous substances is increased. A certain increase in the relative content of these substances is also observed in roots, but still there is less absolute content in the roots of treated plants than in the roots of control plants. Since the total amount of nitrogen in treated plants is almost not increased, it should be admitted that protein synthesis in these organs takes place at the expense of nonprotein substances flowing from the leaves. This is well illustrated by the numerical data of Table 3.

The cause of the indicated redistribution of the nitrogenous substances lies, it seems to us, in an increase in meristematic activity of stem tissues. Here we evidently encounter the phenomenon which is observed also during the action of growth stimulators on rooting plant cuttings [55-57].

Therefore, one of the characteristics of the herbicide action on beans, along with the retardation of nitrogen absorption and inhibition of protein synthesis, is the flow of substances from the leaves into the roots and stems. The results we obtained are in complete agreement with the data present in the literature touching on the effect of 2,4-D on beans and other dicotyledons [7, 13, 28].

As our experiments have shown, the treatment of oat plants with 2,4-D solution did not have any significant effect on the content and interrelationship of separate forms of nitrogen in the whole plant, as well as its separate organs. Therefore, we will not give all those experimental data, but, as a characteristic example, we shall point out only the fact that, in the oat plant treated with 2,4-D at the 3-leaf stage, the content of total nitrogen on the fourth day after treatment was 667 mg (per 100 plants), while in the control plants, it was correspondingly 651 mg. An analogous picture was also observed in plants treated at the 5-leaf stage.

Infiltration with 2,4-D solution had caused basically the same disturbances in the metabolism of nitrogenous compounds as took place after spraying bean plants with herbicide solution. Namely, under the influence of the infiltration treatment, nitrogen uptake by the plants from the nutrient medium was interrupted, the protein content was decreased, and the content of soluble nitrogenous substances was significantly increased.

TABLE 4

Effect of 2,4-D on Synthetic and Hydrolytic Protease Activity in Leaves and Stems of Beans

Experimental treatment	Synthetic activity in mg N per g dry weight per hour			Proteolysis in % of total N for 40 hours			Proteolysis in mg of nonprotein N/g dry wt. for 40 hours		
	1*	4*	8*	1	4	8	1	4	8
Leaves									
Control	3.36	3.45	4.12	4.0	6.0	4.5	0.23	0.39	0.27
0.1% solution of 2,4-D	0.0	0.0	0.0	11.5	10.2	13.5	0.79	0.82	0.65
0.02% solution of 2,4-D	0.0	0.0	0.0	—	—	—	—	—	—
Stems									
Control	—	—	—	17.0	12.6	19 5	0.39	0.27	0.37
0.1% solution of 2,4-D		—	—	43.0	22.5	12.8	1.04	0.74	0.64

*Samples for the measurement of protease activity were taken after 1, 4, and 8 days.

We also analyzed plant material from this treatment method for amino acids, amides, residual nitrogen and ammonium content. From Figure 1 it can be seen that the increase in nonprotein nitrogen in the first days after treatment takes place at the expense of amino acids, amides, and residual nitrogen content. Accumulation of amino acids should be considered to be a result of the hydrolytic breakdown of protein. The enrichment of tissues by amides indicates that the remaining amino acids undergo deamination, and that the ammonia thus produced is used in amide synthesis [58]. This allows one to assert, in turn, that the treated plants (at least in the first eight days after infiltration with 2,4-D solution) do not lose their capacity for removing the ammonia which splits off during deamination by transferring it to amide nitrogen.

As can be seen from the data given, the content of amino acids, amides, and particularly of residual nitrogen is also increased in control plants, but the source of this increase is completely different – this is a synthetic transformation of simple nitrogen compounds in the plants.

The data we obtained concerning the content of nitrogenous substances in separate organs of beans gave, in principle, the same results when plants were sprayed with 2,4-D solution. Particularly, it was found that the leaves of infiltrated plants were continuously depleted in protein, and that the breakdown products of the latter flow out of the leaves into the other plant parts. In the stems, the amount of protein and nonprotein nitrogen is considerably increased as a result of the inflow of nitrogenous substances from the leaves. However, the treated plants were left far behind the control plants in the absolute content of protein nitrogen, which is evidently connected with a faster cessation of meristematic activity of stems after infiltration. In roots, during all these periods of sample-taking, the content of the separate nitrogenous forms remained approximately at the same level, while in the root of controls, the content of these substances increased.

In Table 4 the results of protease activity determinations in treated and control bean plants are given.

TABLE 5

Content of Various Forms of Nitrogen in Oat Plants After Infiltration With 2,4-D Solution (at the 3-leaf stage)

Time of sampling	Treatment	Total N		Protein N		Nonprotein N	
		in mg per 100 plants	in %	in mg per 100 plants	in %	in mg per 100 plants	in %
After 2 days	Control	622	100	525	84.5	97	15.5
	2,4-D	517	100	422	81.7	95	18.3
After 4 days	Control	953	100	775	81.3	178	18.7
	2,4-D	628	100	502	79.8	126	20.2
After 8 days	Control	1230	100	992	80.6	238	19.4
	2,4-D	770	100	579	75.2	191	24.8

From the data of Table 4, it can be seen that infiltration of 2,4-D solution leads to a rapid breakdown of synthetic capacity and increase in hydrolytic activity in bean leaves. Insofar as the hydrolytic activity of protease in stem tissues is concerned, it is also significantly increased. However, on the fourth day and particularly on the eighth day, a decrease in proteolysis takes place. The cause of this phenomenon must evidently be sought in the fact that, along with the increase of protein hydrolysis in several sections of the stem, an activation of meristematic activity, and therefore an increase of protein substance activity, was observed.

On the basis of the data considered, it may be concluded that the equilibrium in proteolytic enzyme activity is irreversibly broken in bean plants under the influence of 2,4-D.

In Table 5 are given the data concerning the content of various forms of nitrogen in oat plants infiltrated (at 3-leaf stage) with a 2,4-D solution. As the data show, oat plants react to infiltration with relatively significant changes in the metabolism of nitrogenous substances. In the treated plants, the content of total and protein nitrogen is sharply decreased, while the accumulation of proteins is retarded to a somewhat greater degree than is the process of nitrogen uptake. Because of this, an increase in the nonprotein nitrogen part takes place, but the absolute amount of nitrogen is still lower in the treated plants than in the control plants. The

TABLE 6

Content of Various Forms of N (in mg) in Separate Organs of Oats After Their Infiltration With 2,4-D Solution (at the three-leaf stage)

Time samples taken	Experimental treatments	Leaves						Stems						Roots					
		Total N		Protein N		Nonprotein N		Total N		Protein N		Nonprotein N		Total N		Protein N		Nonprotein N	
		per 100 plants	per g dry wt.	per 100 plants	per g dry wt.	per 100 plants	per g dry wt.	per 100 plants	per g dry wt.	per 100 plants	per g dry wt.	per 100 plants	per g dry wt.	per 100 plants	per g dry wt.	per 100 plants	per g dry wt.	per 100 plants	per g dry wt.
Second day	Control	370	52.2	320	45.13	50	7.07	121	40.5	93	31.20	28	9.30	131	23.2	112	19.80	19	3.30
	2,4-D	284	49.0	237	40.84	47	8.16	94	41.7	70	30.89	24	10.81	139	24.0	116	19.96	23	4.04
Fourth day	Control	470	53.7	399	45.60	71	8.10	193	48.2	142	35.50	51	12.70	290	26.4	234	21.30	56	5.10
	2,4-D	327	44.5	268	36.50	59	8.00	125	34.1	86	23.30	39	10.80	176	25.2	148	21.20	28	4.0
Eighth day	Control	650	33.2	559	28.55	91	4.65	244	19.7	181	14.60	63	5.10	336	18.5	251	13.85	85	4.65
	2,4-D	372	38.0	284	29.00	88	9.00	148	28.8	97	18.95	51	9.85	250	27.2	197	22.03	63	5.87

enrichment of plants with nonprotein nitrogen takes place mainly at the expense of amino acid, amide, and residual nitrogen (Figure 1). The content of ammoniacal nitrogen is left almost unchanged.

From Table 6 it follows that an increase in all analyzed forms of nitrogen takes place in all organs of the treated plants. The increase in content of nonprotein nitrogen in the infiltrated oat plants, as well as in controls, takes place at the expense of amino acids, amides, and residual nitrogen, while the relative content of these forms turns out to be higher in the treated plants (Figure 2 and Table 6).

It is characteristic that no redistribution of nitrogenous substances between separate organs of plants takes place during the action of 2,4-D in oat plants (Table 6).

Therefore, the nature of the action of 2,4-D on the distribution of nitrogenous substances between separate organs of oat plants is basically distinguished from their distribution in beans. The cause of such a great difference should evidently be sought in the characteristics of the metabolism and of the anatomical construction of these two species of plants.

From the data of Table 7, it follows that in oat plants infiltrated with 2,4-D solution the synthetic activity of protease decreases relatively slowly, while its hydrolytic activity is noticeably increased in stems and almost completely insignificantly so in leaves.

Consequently, insofar as the action of 2,4-D on the synthetic and hydrolytic activity of proteases is concerned, oat plants are also sharply different from bean plants.

The data obtained indicate that, insofar as the action of 2,4-D is concerned, the proteases of oat plants are more stable than the proteases of bean plants. However, as a result of prolonged existence in a state of extreme inhibition of synthetic activity, oat plants treated in this phase are incapable of normal growth, and the majority of them die out.

It is characteristic that the interrelationship of protein to nonprotein nitrogen in oat plants infiltrated with more dilute solutions of 2,4-D (0.01%, 0.025%, and 0.05%) is re-established up to the level of control plants.

Therefore, the analysis of data obtained with infiltration shows that the plants investigated give sharply different reactions to the introduction of 2,4-D. In beans, an irreversible injury in the metabolism of nitrogenous substances takes place: the uptake of nitrogen is interrupted, the process of protein formation is rapidly and completely suppressed, the processes of breakdown greatly increase, and the normal equilibrium of exchange of nitrogenous substances between various organs of plants is broken. It is natural that, with this sharp decrease in synthetic functions and the simultaneous increase in hydrolytic breakdown, the plant becomes incapable of further growth and inevitably dies.

Oat plants, as distinguished from bean plants, respond to the action of 2,4-D by a weaker breakdown in the transformation of nitrogenous substances.

TABLE 7

Effect of 2,4-D on Synthetic and Hydrolytic Activity of Proteases in Oat Leaves and Stems of Oats Plants

Treatment	Synthetic activity in mg N per g dry weight per hour			Proteolysis					
				in % of total N for 40 hours			in mg of nonprotein N per g dry weight per 40 hours		
	1*	4*	8*	1	4	8	1	4	8
Leaves									
Control	5.2	4.84	5.92	11.0	12.5	13.0	0.58	0.52	0.57
2,4-D solution, 0.1%	5.4	2.04	0.84	9.5	12.5	14.4	0.39	0.55	0.68
Stems									
Control	—	—	—	20.7	24.1	25.3	0.90	1.25	1.49
2,4-D solution, 0.1%	—	—	—	25.5	28.5	34.8	0.94	1.45	1.85

* Samples for determination of proteases were taken after 1, 4, and 8 days.

Comparing our experimental data with the material existing in literature [24, 33, 59, 60], we conclude that the attempt of a number of authors [42, 61-63] to explain the different reactions of dicotyledons and cereals to the substances of the 2,4-D type of anatomical-morphological characteristics of these plants alone, is a one-sided concept, and narrowly defines the problems of selective toxicity.

Of course, the anatomical-morphological characteristics of the plants concerned (leaf structure, distribution of meristems, the nature of growth, etc.) have a definite part in their reaction of 2,4-D; however the significance of these characteristics is often interpreted in an exaggerated way. Thus, Gallup and Gustafson [51] suggest that the meristems localized in the nodes of the stems and at the base of leaves in cereals serve as peculiar barriers in the transport pathway of 2,4-D, which can essentially affect the distribution of herbicide in the plant. However, this is contradicted by the fact that cereals, even in the young stages (until stem elongation), are characterized by a considerable resistance to 2,4-D, although at this period they are relatively similar to dicotyledons, at least in the distribution of meristems. In addition, if different resistances of plants to 2,4-D were determined only by the characteristics of meristem distribution and the nature of growth, then the differences in reaction of young plants to the infiltration of 2,4-D solution, which we have, would remain unexplained. Because of this, we consider that the different resistances of cereals and dicotyledons to substances of the 2,4-D type represent a complicated phenomenon conditioned by the entire combination of physiological and anatomical-morphological characteristics of the plant group concerned. The decisive part here is evidently played by the different capacities of the plants being compared to detoxicate the herbicide and to overcome the cause of disturbances in metabolism [36, 38, 40, 64].

It seems to us that the further investigations in the field of the study of the selective action of 2,4-D, as well as of other herbicides, should be directed above all to the clarification of the mechanism of their toxic action and of the ways in which they are detoxicated in different plants.

SUMMARY

Doses of 2,4-D which act as herbicides on bean plants only weakly affect oat plants. 2,4-D stops nitrogen uptake from the nutrient medium, sharply weakens synthesis and enhances decomposition of proteins, completely paralyzes the synthetic activity of proteases and increases the hydrolytic activity of these enzymes. Doses of 2,4-D furthermore enhance the flow of nitrogenous substances from leaves to stems and roots, whereas in oats the same doses produce only insignificant and transient changes in the uptake and transformation of nitrogenous substances.

In contrast to beans, in the oat plant the synthetic activity of proteases gradually decreased, while their hydrolytic activity gradually increased. As a result in oats the shift of metabolism of nitrogenous substances towards hydrolysis was smaller. As in the case of the bean plant, the uptake of nitrogenous substances in oat

plants did not cease; the rate of this process only decreased. The largest shift of nitrogen metabolism in oat plants in the direction of hydrolysis was observed during the most sensitive phase (third-leaf stage).

The fact that disturbance of nitrogen metabolism in oat plants is less pronounced and is caused only by higher concentrations than those for the bean plant signifies that oat plants are more resistant against 2,4-D and that cereals are more capable of detoxication of 2,4-D.

Anatomical and morphological differences in dicotyledonous and monocotyledonous plants are not sufficient to explain the difference in their resistance to substances of the 2,4-D type. The difference in the reactions of these plants upon application of 2,4-D is determined by the anatomical and morphological as well as physiological peculiarities, the decisive role being played by peculiarities in metabolism, including the effect of 2,4-D on metabolism of nitrogenous substances.

The different nature of disturbances in nitrogen metabolism in the investigated plants is one of the main internal factors responsible for stability of the plants with respect to 2,4-D.

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CONCERNING THE ACCUMULATION OF CHLOROPHYLL A AND B IN THE LEAVES OF YOUNG APPLE SEEDLINGS

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Since the time of the classical work of Tsvet, and later, of that of Wilstätter and Stoll, who obtained chlorophyll in the chemically pure state, there has existed the possibility of the absolute quantitative determination of chlorophyll. Such a quantitative determination, at first visual-colorimetric, and then photoelectric, permitted one to pose and to solve a large number of problems connected with the biology of chlorophyll, and in particular, with the course of its accumulation in the ontogenesis of different plants. Several hundred separate essays and several monographs (Giubbenet [1], Rabinowitch [2, 3] and others [4, 5]) can already be enumerated in this field.

However, most of these works concern only changes in the total amount of chlorophyll (a + b) [6, 7]. In only a few works have measurements of the amounts of each component separately been carried out, yet systematic study of the course of accumulation of chlorophyll a and b in ontogenesis under different external conditions is of considerable interest.

Data about the course of accumulation of chlorophyll a and b during a systematic study in the ontogenesis under various conditions, undoubtedly must give information concerning the nature of the interrelationship of both components, both in relation to the mechanism of their formation and in relation to their functional significance. However, there have not been any systematic observations of the accumulation of chlorophyll a and b at various times of growth.

Willstätter and Stoll [8] found a relatively constant interrelationship of components a and b in most cases; at the same time, however, they observed noticeable deviations from this rule, even when individual deviations in various systematic groups, especially in algae, were not taken into account (changes of the ratio a to b were observed in several dozen cases). Later, Hans Fischer [9], Seybold and Egle [10], and others, found component b to be completely absent in several algae.

In addition to the effects of the genetic factor, the ratio of the two components is undoubtedly influenced by various external factors, especially light, which affects the total chlorophyll content as well as the ratio of its components. Variation in the ratio a to b, although considerably smaller, is still observed in the leaves of the same plant if they are placed in the same conditions. Wilstätter and Stoll found that these variations were relatively small, and at the same time they confirmed the observations of Liubimenko [11, 12] concerning a greater chlorophyll content at lower illumination.

In addition, a certain increase in component b content was observed in shaded leaves of the same plant. The comparative constancy in the ratio of chlorophyll a and b had led these authors, in their early work, to a very interesting hypothesis of the functional significance of the chlorophyll components.

*Russian translation.

Chlorophyll a reduces carbon dioxide directly or indirectly while being transformed into component b, after which chlorophyll b is reduced by carotene which is transformed into an oxygen-containing product– xanthophyll; finally, xanthophyll gives up oxygen to the air; this cycle is repeated continuously many times under the action of light, which supplies energy. This orderly hypothesis did not, however, receive confirmation and the authors themselves later abandoned it.

Even under conditions of illumination exceeding that of full sun, and a carbon dioxide content increased by several percent, no noticeable deviation was observed in chlorophyll a and b content or ratio, which would inevitably have taken place if there actually were a mobile equilibrium between chlorophylls a and b, carotene, and xanthophyll; under conditions of such sharp variations of illumination the equilibrium would undoubtedly have been displaced.

In their latest work, Wilstätter and Stoll [13] proposed another hypothesis explaining the significance of the presence of two chlorophyll components in plastids. Comparing the spectra of the two components, the authors point out that they mutually complement each other in light energy absorption, allowing the photosynthetic apparatus to utilize some energy in a considerably greater degree. This idea received unanimous support and confirmation in the work of later authors, particularly in the numerous works of Seybold and Egle, who supplemented it with the fact that chlorophyll a and b possess considerable lability, so that the amount and ratio of components changes under the influence of external factors, evidently changing often so that complete absorption takes place under the conditions of the given light regime.

Seybold and Egle [14] had found that plants at high light intensity are distinguished by a predominance of chlorophyll a, that is the ratio of a to b $\approx$ 5; such are, for example, the plants of open meadows and steppes (in Thymus angustifolius the ratio of a to b is 5.8, in Artemisia campestris is 3.6), and also aquatic plants with leaves on the surface of the water (in Aponogeton distachyum the ratio of a to b 5.5).

Plants slightly shaded, receiving predominantly diffuse light, with a somewhat greater content of chlorophyll b have a ratio of a to b of about 3. In greatly-shaded plants, as, for example, those which grow under the forest canopy, the ratio of a to b decreases down to 2 and even lower; in this group may be included also those aquatic plants with leaves which are distributed at considerable depths (in Ceratophyllum emersum the ratio of a to b is 2.9 at depths of 0.5 m, and in Elodea canadesis it is 1.65 at depths of 2 m).

Considering the experiments of Wilstätter and Stoll with woody plants mentioned earlier, the authors come to the conclusion that the increase in the relative content of chlorophyll a in light-exposed (top) leaves of chestnut and elm which was found in these experiments is particularly interesting, because the change of chlorophyll content is found here in the leaves of the same plant, and therefore the genetic factor is excluded. This change may be seen, undoubtedly, as an ontogenetic adaptation. Insofar as the small value of these deviations is concerned, we reached the conclusion that these were due to the comparatively small difference in illumination; and that the "lighted leaves" of Wilstätter and Stoll were really moderately illuminated, i.e., they referred to the second category of illumination, according to the characteristics of "lighted field," proposed by Seybold.

In the experiments of Seybold and Egle with different trees, chromatographic analysis of the pigments of the upper well-lighted leaves and of those distributed in the deep lower part of the crown showed significantly greater changes in the amount of ratio of a to b in the well-lighted and shaded leaves. Thus, the ratio of a to b in Sambucus nigra was 3.6 in well-lighted (top) leaves, and 2.6 in shaded leaves; in Asculus hippocastanum it was 4.85 in well-lighted leaves and 3.4 in shaded leaves; in Fagus silvatica it was 3.3 in well-lighted leaves and 2.5 in the shaded leaves; in Phaseolus multiflorus it was 4.5 in the lighted leaves and 3.3 in the shaded leaves. Therefore, the relative increase of chlorophyll b content in shaded leaves may amount to 40%, and this increase evidently represents adaptation to the low illumination.

In the recent work of Wolft [15], an investigation was made of the changes in ratio of a to b in green leaves and in leaves that have partially lost their chlorophyll during the autumn dying. As a result of an analysis of chlorophyll a and b in completely green leaves and in leaves that were just beginning to turn yellow, in thirty woody species, it was established that leaves that are beginning to lose chlorophyll always have a smaller ratio of a to b, as compared to the green leaves of the same tree. Chlorophyll b is more stable in the process of autumn pigment destruction.

EXPERIMENTAL PART

The study of the problem of accumulation of pigment components in relation to the illumination of fruit plants during their ontogenesis has both theoretical and practical significance. In this connection, we posed the

problem of clarifying the effect of light regime on the content of the separate components, chlorophyll a and b, since their ratio, according to the, as yet, relatively few investigations, is a reflection of the adaptation of the pigment system to the conditions of illumination.

Seedlings of wild apple of the first and second year of growth were taken as experimental objects. The seeds were stratified in the winter. In the beginning of May, plants at the stage of cotyledons and the rudiment of the true leaves, were planted in the ground on allotments in duplicate. Planting was done at a distance of 20 cm apart in the row and 20 cm between rows. Definite light conditions were established from the moment of planting. Part of the planting was shaded with cheesecloth to 50% of full illumination, another part was shaded to 25%, and the third part, for a control, was left on full illumination.

Sampling was done according to the method we described earlier [16].

Acetone extractions of leaf pigments were brought to a certain volume and were measured spectrophotometrically on an SF-4 spectrophotometer.

The amounts of chlorophyll a and b were determined according to the following equations:

$$Ca = 10 \log \frac{I_0}{I(662)} - 0.8 \log \frac{I_0}{I(643)};$$

$$Cb = 17.6 \log \frac{I_0}{I(643)} - 2.81 \log \frac{I_0}{I(662)},$$

where C – mg of chlorophyll per liter, I_0 – the incident light intensity, I – the transmitted light intensity.

The experimental results are given in the Tables 1 and 2.

TABLE 1

Amount of Chlorophyll A and B in Growing Leaves of First-Year Apple Seedlings (in mg chlorophyll per 10 g of fresh weight of leaves)

Date	Days of growth	Lighted				Slightly shaded (75% illumination)				Strongly shaded (50% illumination)			
		a	*b*	*a+b*	*a : b*	*a*	*b*	*a+b*	*a : b*	*a*	*b*	*a+b*	*a : b*
6/13	10	5.70	2.04	7.74	2.8	5.60	1.84	7.44	2.8	5 86	2.16	8.02	2.7
6/18	15	8.51	2.90	11.41	2.9	8.82	3.30	12.12	2.7	9.15	3.48	12.63	2.6
6/22	19	19.75	7.12	26.87	2.8	22 20	7.75	29,95	2.8	22.45	8.13	30.58	2.7
6/28	25	25.58	8.03	33.61	3.2	29.45	10.12	39.57	2.9	31.23	12.01	43.24	2.6
7/4	32	24.75	7.98	32.73	3.1	31.02	11.02	42.04	2.8	35.61	13.27	48.88	2.6
7/9	37	27.81	8.39	36.20	3.2	34.22	12.03	46.25	2.7	37.52	15.08	52.60	2.5
7/12	40	33.03	11.02	44.05	3.0	38 65	13.61	52.26	2.8	47.22	18.10	65.32	2.6
7/18	46	32.00	10.70	42 70	3.0	37.86	12.85	50.71	2.9	42.50	15.80	58.30	2.7
8/25	84	40.20	13.39	53 59	3.0	51.64	18.02	69.66	2.8	50.25	21.15	71.40	2.5
9/12	102	24.38	8.76	33.14	2.8	34,02	15.20	49.22	2.4	32.89	14.90	47.79	2.2
10/10	130	18.15	7.00	25.15	2.6	24.12	12.03	36.15	2.0	23.05	12.60	35.65	1.8

As can be seen from Table 1, in spite of relatively significant differences in illumination, the changes of the ratio of a to b are relatively small, while at the same time, they are systematic in nature. With a decrease in illumination, the amount of component b is increased; this increase is correlated with the degree of decrease in illumination. This predominance of component b is greater in the well-shaded (50%) leaves than in the slightly shaded leaves. In the process of pigment accumulation during ontogenesis, this predominance of component b increases more and more according to the degree of leaf development, and is especially noticeable in the end of growth. In intensely shaded (60%) plants, the ratio of a to b is equal to that of the control, which is 2.8; towards the end of growth, it decreases to 1.8, and in slightly shaded (up to 25%) plants, it is decreased from 2.8 to 2.0, while the ratio of a to b remains almost constant in the control plants throughout the entire growing season.

A similar correlation of chlorophyll components a and b is also observed in two-year-old apple seedlings, as can be clearly seen in Table 2.

TABLE 2

The Amount of Chlorophyll A and B in Growing Leaves of Two-Year-Old Apple Seedlings (in mg chlorophyll per 10 g of fresh weight of leaf)

Date	Lighted leaves				Slightly shaded (75% illumination)				Strongly shaded (50% illumination)			
	a	*b*	*a+b*	*a : b*	*a*	*b*	*a+b*	*a : b*	*a*	*b*	*a+b*	*a : b*
6/13	9.30	3.20	12.50	2.9	9.80	3.52	13.32	2.8	9.10	3.22	12.32	2.8
6/18	13 03	4.80	17.83	2.9	14.43	5.20	19.63	2.7	13.88	4.85	18.73	2.8
6/22	17.39	5.85	23.24	3.0	19.79	7.08	26.87	2.8	19.57	7.80	27.37	2.6
6/28	20.89	7.52	28.41	2.8	23.28	9.55	32.83	2.6	22.62	8.73	31.35	2.5
7/4	26.20	8.42	34.62	3.1	31.10	11.05	42.15	2.8	33.53	12.30	45.83	2.7
7/9	24.25	8.11	32.36	3.0	30.60	10 55	41.15	2.9	32.08	12.06	44.14	2.6
7/12	28.19	9.73	37.92	3.0	35.84	12.83	48.67	2.8	36.66	15.05	51.71	2.7
7/18	32.73	11.06	43.79	2.9	37.11	13.34	50.45	2.7	38.04	15.58	53.62	2.5
8/25	36.41	12.51	48.92	2.9	42.50	15.30	57.80	2.7	45.86	19.01	64.87	2.4
9/12	18.42	6.60	25.02	2.8	20.08	8.73	28.81	2.3	23.88	15.18	39.06	1.6
10/10	14.45	6.01	20.46	2.4	16.02	8.20	24.22	1.9	16.30	12.67	28.98	1.3

SUMMARY

The aim of the present investigation was to study the dynamics of accumulation of chlorophyll a and b in leaves of annual and biennial apple seedlings under various light conditions.

A definite ontogenetic adaptation was found regarding the general increase of total chlorophyll as well as relatively greater accumulation of chlorophyll b.

In experiments with annual seedlings darkening was found to affect the ratio of a to b at a period close to termination of the vegetative period. Thus the ratio of a to b of the plants grown in full sunlight was 2.6 whereas the ratio of a to b of the plants grown at 25% of full sunlight was 2.0, and the ratio of those at 50% full sunlight was 1.8.

The a/b ratio of the biennial seedlings exposed to full sunlight was 2.4; the ratio of the plants at 25% full sunlight was 1.9, and the ratio for those at 50% was 1.3.

The relative increase of the b component was apparent, to a smaller degree, already at early stages of development and progressively grew, attaining the mentioned values at the termination of the vegetative period.

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THE EFFECT OF GROWTH PROCESSES ON THE CAPACITY FOR HARDENING

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We have studied the hardening of tissues in winter cereals with the aid of sugar absorbed from an external solution [1]. For this purpose, we developed and used a laboratory method, using coleoptile sections. This method allows one to carry out physiological investigations of a number of other phenomena connected with the frost resistance of plants. In the present work, this method was used for the clarification of the dependence of the degree of hardening upon the rate of growth processes. The incompatibility of growth and resistance represents one of the basic principles in the field of cell physiology [2]. Coleoptiles turn out to be convenient objects, both for the measurement of increasing growth, as for the determination of frost resistance in the tissue.

Suppression of Growth Processes During Hardening

The necessity of low temperature for hardening cells lies first and foremost in the weakening of plant growth. In this way, a physiological state is created which is favorable for the development of the properties of frost resistance in the protoplast. To prove the existence of such a state, an experiment was set up in which resistance was measured after different growth rates during the hardening period.

For variation in growth rates, the experiment was carried out at different constant temperatures: +0.5; +3.0 and varying temperatures (+7.8 in the day time, and +2.7 at night).

Under these conditions, coleoptile sections of winter rye, variety viatka, passed the first phase of hardening in darkness on a 12% sucrose solution for a period of 8 days. Afterwards, the sections were transferred into a refrigerator at a temperature of −3.5° for three days (the second phase of hardening); then the percent of survival during dangerous frosts was determined.

The lower the temperature, the weaker was the growth of the tissue (Table 1). At +0.5°, the growth increase was 17%, but it increased up to 56% at +3.0°, and with the daily warming, it reached 93%. The dependence of frost resistance of the sections on the rate of growth processes was observed. The greater the increase in growth during the first phase, the weaker was the hardening of the cells. For example, at −16° the percent of surviving coleoptiles was 100 in the first treatment, 63 in the second, and in the third they all died. In all cases, the tissues had a sufficient amount of protective substances (the coleoptiles were kept on 12% sucrose solution). From the experiment described it can be seen that this unfavorable effect on the frost resistance of cells is exerted by rapid growth. Growth supression was thus obtained by means of keeping the sections at 0°.

TABLE 1

Growth Increase and Frost Resistance of Cylinders Cut From Coleoptiles of Winter Rye, Variety Viatka, During Their Hardening at Various Temperatures on a 12% Sucrose Solution

Temperature of the first phase of hardening	Growth increase in % for the hardening period	Percent of living coleoptiles after treatment with below-zero temp.			
		−9 to −10°	−11 to −12°	−14 to −14.6°	−16 to −16.4°
+0.5	17	100	100	100	100
+3.0	56	100	100	94	63
+7.8 during day and +2.7 at night	93	77	15	35	0

The weakening of growth processes during hardening is obtained not only by the action of low above-zero temperature, but also by accumulation of sugars in the tissues. For proof, we give measurements of the increase in growth of coleoptile sections of winter rye, variety Viatka, for the period of the first phase on various sucrose concentration:

Percentage of sucrose in external solution	Increase in growth in % for the period of the first phase of hardening	
	at + 3°	at 0°
4	80	54
12	32	16

From the data obtained, it can be seen that the rate of growth processes in coleoptiles decreases to one-third with an increase in the concentration of the external sucrose solution, to the value optimal for hardening (12%). We established earlier [1] that coleoptiles kept on such a sucrose solution accumulated 11% of sugars, i.e., almost as much as there was in the surrounding nutrient medium. From a comparison of these data, it follows that growth decreases as a result of enrichment of the cell contents with reserve substances. Under natural conditions, an accumulation of sugars in the hardened plants may contribute to the fact that their growth is noticeably retarded during warm periods, and the frost resistance under such conditions is least decreased. It should be emphasized that the accumulation of sugars in cells is a greatly contributing factor, since the growth increase is slowed down under its effect, and at such a low temperature as 0°.

Tumanov [3] has established that the first phase of hardening in green winter cereals may take place only in the light with an accumulation of sugars. Because of this, the role of light in this process is usually summarized only as the accumulation of reserve substances through photosynthesis. For confirmation of this, the experiments of Dexter are cited [4]. He hardened winter plants in the light, but in an atmosphere lacking carbon dioxide, and he did not obtain an increase in frost resistance. The set-up of this experiment, however, was such that one can not conclude from it that light contributes to the increase in resistance only through photosynthesis. In this case, enrichment with reserve substances was excluded. The latter were even decreased as a result of their expenditure for growth. Because of this, we took for experimental objects the colorless, chlorophyll-lacking, organs—coleoptiles. The enrichment of these tissues with reserve substances was accomplished by the absorption of sucrose from an external solution. All this allowed us to clarify the question: does light play any particular role during hardening of the tissues except through photosynthesis?

The first phase of hardening in coleoptile sections in our experiment took place on 12% sucrose solution for the period of 8 days in the open air in the fall. In one treatment, the sections were exposed to daylight illumination, in another, — to darkness (the small containers of coleoptiles were covered with black paper, and then with white paper on top). The average temperature was 7.8° in the day time and 2.7° at night. Afterwards, the coleoptiles, as in all our other experiments, passed the second phase of hardening at 13.5° for a period of

three days under similar conditions. After that, the frost resistance of the experimental objects was determined. The results of the experiments are given in Table 2.

TABLE 2

The Effect of Daylight Illumination During the First Phase of Hardening on Frost Resistance of Coleoptiles of Winter Rye, Variety Viatka

The first phase of hardening	Growth increase during the time of hardening, in %	Percent of living coleoptiles after treatment at:			
		−9°	−12°	−14.5°	−16.5°
In the light	16	100	47	50	0
In darkness	93	77	15	35	0

The growth of isolated coleoptile cylinders was significantly less during hardening in the light (16%) than in darkness (93%). In correspondence with this, the frost resistance of the experimental objects in the first case was considerably higher than in the second. Since photosynthesis was excluded in our experiment and the accumulation of sugars by the cells was insured by their absorption from the external solution, it then follows from the data obtained that light may influence the frost resistance not only through photosynthesis, but by way of inhibition of growth processes.

However, this role of illumination is not great; it is noticeable only during temperatures unfavorable for hardening, and while the accumulation of sugar in the cells is not yet reached. This conclusion follows from another experiment of ours in which the first phase took place at a lower temperature (+0.5° for nine days, also on 12% sucrose solution). In this case, the coleoptiles were exposed daily to day light, but only for one hour. It is known that such a short period of light action is sufficient to produce growth curvatures in sprouted cereals.

In the experiment described, the growth increase in coleoptiles and their frost resistance (up to −16.4°) were the same in both treatments. These two factors − low temperature (+0.5°) and 12% sucrose solution − suppress growth processes to such a degree that the effect of light already becomes noticeable under these conditions. It follows from this that radiant energy does not exert any specific action on protoplasm during hardening. It is involved either through photosynthesis, contributing to the accumulation of reserve substances, or by changing the rate of growth processes. Under natural conditions, light may play a noticeable part only in the beginning of hardening, and then only during insufficiently cold weather.

In frost-resistant woody species, the suppression of growth occurs usually in the end of the summer under external conditions which are still favorable, by means of the fact that meristematic tissues of the aerial part enter into the dormant state. Winter plants do not have that capacity. The latter attain a slowing down of the rate of growth processes only during the first phase of hardening under the influence of light, lower temperatures, and accumulation of sugars. The data given show that the retardation of growth is one of the necessary conditions for the successful occurrence of the process of hardening and the development of high frost resistance in plants.

It does not follow, however, from the above statements that the retardation of growth processes, alone is sufficient for the increase of frost resistance in winter plants.

In our experiment, the first phase of hardening of winter rye coleoptiles, variety Viatka, was passed for nine days at 3° on a 12% solution of sucrose and on distilled water.

The increase in growth on distilled water happened to be less than half that of the plants on 12% sucrose solution (25% in the first case versus 60% in the second case). In spite of such a sharp lowering of growth rate, the frost resistance in the first case was not measurably lower than in the second. The analysis show that during the hardening of coleoptiles on distilled water, not only is no accumulation of sugars observed, but the sugar concentration is decreased because of the expenditure for growth and for respiration [1]. From this experiment, it follows that the frost resistance of plants cannot be increased by suppression of growth processes alone without

enrichment of cells with reserve substances. Slowing-down of their growth turns out to be an important factor in the process of hardening, but not the only one by far. Because of this, cases of disagreement between growth rate and resistance cannot be used as proofs of absence of a close connection between these phenomena.

It may be suggested that the higher the temperature during the germination of plants and during coleoptile formation, the more intense are the growth processes in the tissues, and the harder it is to suppress the growth during hardening. As a result, one would expect a lower resistance in coleoptiles growing under favorable temperatures. The experiment we set up, in which the effect of growth temperature on frost resistance of coleoptiles of winter rye, variety Viatka was studied, confirms such a dependence:

Growth temperature	Percentage of coleoptiles alive after treatment with below-zero temperatures	
	−9°	−15°
+9°	100	85
+19°	62	0

From the seedlings thus obtained, sections of coleoptiles were prepared, which were then exposed to unfavorable conditions for the first phase of hardening: five days on 4%, and later six days on 8% sucrose solution. In the second phase, the coleoptiles were exposed also in similar conditions at −3° for the period of three days. The percentage of surviving coleoptiles shows that the plants grown at +9° increase their resistance better than those left at +19°. This phenomenon is noticeable when hardening takes place in unfavorable conditions.

After the optimal external conditions for the development of high frost resistance are provided for winter plants, the physiological state of the cells is also important. This was shown by experiments on coleoptiles of different age. The latter were of different lengths, from 7 mm to 60 mm, and they were carefully sorted according to length. After removal of the tip, 5 mm cylinders were cut from the part below the tip and were then exposed to hardening under similar conditions (Table 3).

TABLE 3

Hardening Capacity, on 12% Sucrose Solution at 0.5°, of Coleoptile Cylinders of Different Lengths From Winter Rye, Variety Viatka, and Wheat, Variety Liutestsens 329

Coleoptile length in mm	Rye			Wheat		
	Percentage of surviving coleoptiles after treatment with different below-zero temperatures					
	−9°	−12°	−16.5°	−9°	−12°	−16.5°
5—7	100	100	100	100	100	0
10—12	100	100	100	100	80	3
15—17	100	100	100	100	89	0
20 - 22	100	100	93	100	43	25
25—27	100	100	71	—	—	—
30—32	100	93	50	100	10	—
40—42	100	60	60	100	0	10
50—52	64	0	0	77	0	0
60—62	0	0	0	0	0	0

Only young coleoptiles were capable of good hardening, surviving at −16.5°. The older coleoptiles, 60 to 62 mm long, increased their resistance very poorly: they were completely dead at −16.5°. The rest were intermediate in their reaction.

For establishing the reason for such different behavior by plants of different ages, a special study is necessary. At the present time, it is only possible to propose a hypothesis which must be tested experimentally. In the youngest coleoptiles, as microscopic observations show, the cells have small vacuoles and are small in size. Then the latter begin to grow rapidly and to increase in size. Such a growth characteristic is a factor preventing

the development of frost-resistance properties in plants [2]. The processes connected with increase in the number of cells retard frost resistance to a smaller degree. On this basis, one may suppose that, in coleoptiles of medium length, the hardening capacity decreases as the growth increases.

The reason for poor resistance of adult coleoptiles is less clear. This effect may be produced by the processes of cell aging. The experimental objects are short-lived organs and their tissues do not live long. During aging, the submicroscopic structure of the protoplasm evidently cannot be supported at the normal level, and the adult coleoptiles harden very poorly. Determinations of frost resistance show that, for example, old crops of alfalfa increase their resistance less than young crops [5].

The Effect of Substances Stimulating and Retarding Growth on the Hardening Capacity of the Plant

Since one of the factors which affects the intensity of growth processes is the content of auxins in tissues, it is necessary to understand what happens to these physiologically active substances during the process of hardening. Determinations of free and bound heteroauxin were carried out by the fluorimetric method of Rakitin and Povolotskaia [6], both prior to and after this process. The same consisted of 300-400 coleoptile sections, each 5 mm long. The experiment was done in duplicate. The first phase of hardening continued for eight days at 2°. The results of the experiment are given in Table 4.

TABLE 4

Changes in Indoleacetic Acid Content During the First Stage of Hardening in Detached Coleoptiles of Winter Rye, Variety Viatka (in μg/g of wet weight)

Determination	On distilled water				On 12% sucrose solution			
	Free		Bound		Free		Bound	
	1	2	1	2	1	2	1	2
Prior to the first stage of hardening	3.2	5.9	46.0	32.4	3.5	5.9	56.5	32.4
After the first stage of hardening	3.7	4.7	31.6	25.8	1.7	2.7	25.1	23.8

Note: 1 and 2 are parallel experiments.

The data of Table 4 show that, in detached coleoptiles with the tip cut off, auxins are present in significant amounts only in the bound form; there is a relatively small amount in the free form. Our determinations agree with the data of the literature in which coleoptiles of corn and oats contain free auxin to the amount of about 8% of the total auxin [7]. What, then, happens to the indoleacetic acid during hardening? It is particularly interesting to learn the fate of heteroauxin, which occurs in the free state, which is distinguished by the greatest activity. When coleoptiles pass the first phase of hardening on water, the content of free indoleacetic acid varies towards increase as well as towards decrease. During hardening on 12% sucrose solution, the amount of free auxin is decreased to approximately one-half. Thus, during the transfer from the unstable to the stable state, the tissues are much impoverished in free auxin. This process is in some way connected with accumulation of sugars in cells, since lower temperature alone is not sufficient for it.

During maintenance of coleoptiles at temperatures close to zero, the amount of the bound auxin in them decreases, but this is more clearly noticeable in hardening on 12% sucrose solution than on distilled water. A particularly strong decrease in bound auxin is observed on sugar solution, when it occurs in tissues in large amounts.

In our experiments there could not have been an outflow of auxin from the coleoptile sections into other parts of the plants. Therefore, one may suppose that a breakdown of indoleacetic acid took place during hardening. The decrease in auxin content in roots and in aerial parts of red clover with the approach of winter was observed by Tarchevskii [8].

Therefore, during hardening a decrease in auxin content in the cells takes place in the winter plants. It was also shown that the sugars, besides their own direct protective action on protoplasm, contribute to inactivation of growth stimulants which interfere with the successful occurrence of hardening. In connection with this, the

question arises: How does indoleacetic acid added externally affect the frost resistance of cells, if added in amounts which increase the cell growth?

Since the optimal concentration for the hardening of coleoptiles was a 12% sucrose solution, in our experiments the indoleacetic acid was added to the latter, in amounts of 10 mg per liter. The first stage of hardening was continued for eight days at 0.5°. After this, the experimental objects were thoroughly washed with water, dried with filter paper, and placed under conditions of the second phase of hardening for four days at −3.5°. Then the temperature in the refrigerator was lowered down to critical values, after which the coleoptiles were slowly thawed and kept at a temperature favorable for growth.

The results of the experiment are given in Table 5.

TABLE 5

Effect of Indoleacetic Acid (in a concentration of 10 mg/liter) on Frost Resistance of Coleoptile Sections of Winter Rye, Variety Viatka

First stage of hardening	Increase in growth during hardening, %	Percent of living coleoptiles after treatment at:			
		−9°	−11.5°	−14°	−16°
Experiment I					
On 12% sucrose solution	15	100	97	93	87
The same + 10 mg/liter indoleacetic acid	30	97	50	40	7
Experiment II					
On 12% sucrose solution	18	100	100	100	100
The same + 10 mg/liter indoleacetic acid	78	90	93	93	90

TABLE 6

The Effect of Various Concentrations of Indoleacetic Acid Given During Preparation for the First Stage of Hardening on Growth and Frost Resistance of the Coleoptiles in Winter Rye, Variety Viatka

First stage of hardening	Increasing growth during hardening, in %	Percent of living coleoptiles after treatment at:		
		−10°	−12°	−18°
On 12% sucrose solution	35	99	100	75
The same + 10 mg/liter indoleacetic acid	50	98	100	75
The same + 200 mg/liter indoleacetic acid	20	76	84	8

The results obtained in the first experiment (Table 5) show that indoleacetic acid increases the growth increase two times and sharply decreased the hardening capacity in coleoptiles. After −16°, 87% of control sections have survived, and only 7% of the experimental sections. But the results of such experiments turn out not to be of similar significance. Because of this, let us consider also a second experiment in which there was no considerable decrease of frost resistance in coleoptiles under the influence of indoleacetic acid, although the conditions of hardening were the same as in the first experiment. The reason for such different behavior should be sought in the characteristics of the coleoptiles taken for the experiment. In both cases, the increase in growth during the first stage of hardening was the same (15-18%) in controls, but after addition of 10 mg/liter indoleacetic acid, it turned out to be sharply different. In the first experiment, it was 30%, in the second 78%, i.e., 2.5 times greater. Here we met up with a phenomenon in which increased growth during hardening is accompanied by high frost resistance of cells.

TABLE 7

Short Duration (8 hours) Action of Indoleacetic Acid in a Concentration of 200 mg/liter Given in the End of the First Phase of Hardening on Growth and Frost Resistance of Coleoptile Sections in Winter Rye, Variety Viatka

First phase of hardening	Increase of growth after addition of substance	Percentage of living coleoptiles after treatment at:		
		−10°	−12°	−18°
On 12% sucrose solution	2.2	99	100	75
The same + 200 mg/liter indoleacetic acid	2.3	100	98	0

TABLE 8

The Effect of 2,4-D on Growth and Frost Resistance of Coleoptiles of Winter Rye, Variety Viatka

First phase of hardening	Increase in growth during the hardening,%	Percent of living coleoptiles after treatment at:			
		−10°	−12°	−14°	−16°
	Experiment I				
On 12% sucrose solution	61	100	100	95	63
The same + 10^{-6}% 2,4-D	62	100	100	89	32
	Experiment II				
On 12% sucrose solution	15	100	97	93	87
The same + 10^{-6} %, 2,4-D	10	100	93	63	63

TABLE 9

The Effect of Various Concentrations of Maleic Acid Hydrazide (MAH) On Growth and Frost Resistance of Winter Rye, Variety Viatka (the first phase of hardening was 7 days at + 3°, and the second phase was 3 days at −3°)

The first stage of hardening	Increasing growth during hardening,%	Percent of living coleoptiles after treatment at:		
		−11°	−14°	−17°
On 12% sucrose solution	43	79	67	67
The same + 0.1% MAH	38	76	—	61
The same + 0.5% MAH	26	65	20	24

TABLE 10

The Effect of Various Concentrations of MAH on the Accumulation of Sugars in Coleoptiles of Winter Rye, Variety Viatka, in the Process of the First Phase of Hardening (in %)

Experimental conditions	Amount of ketoses	Amount of aldoses	Sum of ketoses and aldoses, in %
Prior to hardening	3.2	1.4	4.6
After hardening on 12% sucrose solution	13.4	4.3	17.7
The same + 0.1% of MAH	9.2	4.6	13.8
The same + 0.5% of MAH	8.2	3.7	11.9

For explanation of this result, the hypothesis was proposed that the amount of free auxin in cells has an effect on the ability of the plant to pass successfully the second stage of hardening. In the two experiments discussed (Table 5), the same amount of indoleacetic acid was given externally. One may suppose that in the second case, where the increasing growth is 2.5 times greater, more auxins were used for growth and less remained in the coleoptiles. Because of this, the latter had passed the second stage of hardening better. The result may vary also in the fact that the content of free auxin sharply varies in the original coleoptiles; i.e., in one experiment the amount of free auxin prior to hardening was 3.2 μg per gram of wet weight, and in another was 5.9 μg per g.

In our experiments, the first phase of hardening usually continued for 7 to 8 days. After such a long period, a small amount of indoleacetic acid (10 mg/liter), added to 12% sucrose solution once before the hardening, may be used up by coleoptiles both for growth and as a result of auxin breakdown during the accumulation of sugars in cells. In such a case, the experimental objects came to the second stage of hardening impoverished in auxin. It seemed interesting to test the effect of two concentrations of indoleacetic acid: one was that previously used (10 mg/liter), and the second was considerably higher (200 mg/liter).

The results of the experiments are given in Table 6.

After adding indoleacetic acid in the amount of 10 mg/liter, growth during the first stage was increased, but the frost resistance of coleoptiles did not change. When, however, indoleacetic acid was given in amounts twenty times greater, the growth processes became less intense, and the resistance of tissues was decreased. Such results may be explained by the presence of considerable amounts of heteroauxin in cells towards the beginning of the second stage of hardening.

To check the proposed hypothesis, we have tried to approach it in another way: indoleacetic acid was added, not in the beginning, but in the end of the first phase of hardening. The action of the preparation continued only for eight hours. It was expected that during such a short period the physiologically active substance would have time to penetrate into coleoptiles in sufficient amounts, but that it would not be used very much in the processes of growth and breakdown. Being found inside cells in significant amounts, it should have an effect on the successful occurrence of the second phase.

In our experiment (Table 6), 200 mg/liter of indoleacetic acid had decreased the growth of coleoptiles to almost one-half. Because of this, the decrease in frost-resistance of the tissue may be explained by the injury of cells by the introduced substance. But this explanation was not confirmed. The experiment then was set up in such a way that growth during hardening was the same in experimental plants as in the controls; nevertheless, the sharp decrease in resistance took place (Table 7).

The data obtained allow one to suppose that, in the presence of a considerable amount of auxin in the cells, the protoplasm is not able to pass successfully to the second stage of hardening. For confirmation of this explanation, in further experiments it was necessary to carry out parallel determination both of the free auxin content in tissues as well as of the degree of frost resistance.

In addition to indoleacetic acid, other substances were also tested. In this case, the first phase of coleoptile hardening was carried out on 12% sucrose solution, to which 2,4-dichlorophenoxyacetic acid (2,4-D) was added in a concentration of 10^{-6}%.

It can be seen from Table 8 that 2,4-D in a concentration of 10^{-6}% has a small effect on growth during the dirst phase of hardening, and causes a small decrease in frost resistance. This substance also did not have any effect on growth and frost resistance in grapefruit [9].

While studying the effect of growth processes on the hardening capacity of cells, it is necessary to know what effect is shown by substances which retard plant growth. For this, we used maleic acid hydrazide (MAH) in various concentrations. It was added to 12% sucrose solution before the first phase. Out of many experiments, only one is given here for illustration (Table 9).

The weak concentration (0.1%) of MAH somewhat decreased growth and was not reflected noticeably in the frost resistance of coleoptiles. If, however, the increased concentration (0.5%) is taken, a strong retardation of growth and a decrease in cell resistance is obtained. Even stronger solutions completely stop growth processes and cause injury of the tissues.

With the aid of MAH a slight increase of frost resistance in young lemon trees was obtained [10]. In raspberry, resistance of plants towards spring frosts was decreased by this preparation when retardation of growth was great, and at a slight retardation of growth, resistance was increased, but the yield was always decreased [11]. In coleoptiles, it was not possible to obtain positive effects. Our experiments show that growth retardation does not always reflect favorably on plant resistance. In many cases, the retardation of growth processes simultaneously causes a number of other unfavorable changes. For checking of this proposal, we investigated the accumulation of sugars during hardening of the coleoptiles in winter rye, variety Viatka, on 12% sucrose solution with addition of MAH. The results obtained are given in Table 10.

The analysis shows that, in spite of the decrease in growth, a smaller accumulation of reserve substances takes place. This fact confirms the explanation that MAH not only retards growth, but simultaneously has unfavorable effects on other processes which take place during hardening. As a result, instead of an increase in frost resistance, a decrease in frost resistance takes place.

SUMMARY

1. Coleoptiles are convenient for investigation of the dependence of the degree of hardening on the intensity of growth processes.

2. Inhibition of cell growth caused by low positive temperatures is one of the conditions for successful hardening of winter plants. With approach of the temperature to 0°C the growth decreases and the first stage of hardening proceeds more successfully.

3. Growth is suppressed not only by low temperature but by accumulation of sugars in the plant tissues as well. The latter factor appreciably reduces growth even at a temperature as low as 0°C.

4. Light influences frost hardiness of winter plants not merely through the agency of photosynthesis (accumulation of sugars) but by inhibiting the growth processes as well.

5. However, inhibition of growth alone is not sufficient for complete winter hardening of plants; the amount of protective substances in the tissues must be increased and the cells should be in the proper physiological state.

6. Only young coleoptiles harden well. With increase of age their frost hardiness decreases due to intense growth. Frost hardiness of fully grown coleoptiles is still smaller.

7. The amount of free as well as of bound auxin in the tissues of winter plants decreases upon hardening. However, this decrease of auxin is caused not only by low temperatures but by an increase of sugars in the cells.

8. The ability of coleoptiles to harden is appreciably reduced if a high concentration (200 mg/l) of indoleacetic acid is added to a 12% solution of sucrose. The amount of free auxin in the cell influences its frost hardiness. Hence the growth rate cannot always be regarded as a reliable index of the hardiness of the plant.

9. Winter plant hardening cannot be achieved by suppressing growth with maleic acid hydrazide. High concentrations of the latter, diminishing growth processes, simultaneously cause a number of other undesirable effects, such as a decrease of sugar accumulation in the cell.

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LEAF TEMPERATURE AND HEAT RESISTANCE IN CERTAIN CULTIVATED PLANTS

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Under natural conditions injury of plants by high temperatures is of two types: desiccation injury and scald injury. In desiccation injury, the results of efficient isolation and low relative humidity, the leaves dry up as a result of an unbalanced water regime. Such leaves retain their green color at death. Leaves suffering from scald injury develop burned areas from high temperatures and thus acquire a yellow, white-yellow or brown color. The appearance of burned areas on a large part of the leaf may lead to the death of the plant [1-4].

The adaptive reactions of various plants to high temperatures are of different types. Some become more heat resistant because of an intense transpiration activity [5-7], while others are able to withstand high temperatures because of peculiarities of the colloidal-chemical structure of the protoplasm [8, 2].

The investigations of Genkel and Margolina [9] indicate that protoplasmic viscosity plays a significant biological role in the resistance of plants to high temperatures. Thus, the reason for the high resistance of succulents in comparison with other groups of plants, in the opinion of the authors, is the heightened protoplasmic viscosity and the presence of a large amount of bound water. In another paper [10] it was shown that the reproductive organs of the plant, which have a high heat resistance, have a greater protoplasmic viscosity than vegetative organs.

The experiments of Genkel and Tsvetkova [11] also testify to a close relationship between protoplasmic viscosity and heat resistance. Treating sections of the plants under investigation with solutions of calcium salts, they obtained an increase in protoplasmic viscosity. On this basis, the authors proposed a method of increasing heat resistance of plants by pre-sowing treatment of seeds with solutions of calcium salts [12].

Nevertheless, as the work of Genkel and Badanova [13] shows, an increase of viscosity is not necessarily accompanied by an increase of the threshold temperature for coagulation of protoplasmic proteins. Generalizing from their own experiments and data in the literature, they conclude that there are two kinds of viscosity: hydrophilic and structural. The first type is accompanied by an increase in the amount of hydrophilic colloids and bound water and is correlated with heat resistance, while the second, on the contrary, is associated with a decreased heat resistance.

Petinov and Molotkovskii [14] relate the protective reactions elicited by high temperatures to respiratory metabolism. The more resistant plants are characterized by a labile enzyme system which is able to supply the

*See English translation.

TABLE 1

Leaf Temperature, Water Content, and Transpiration Rate in Sunflower var. Zhdanov 8281 Under Natural Conditions

Date	Air temp. in °C	Relative humidity in %	Cloudiness in arbitrary units	Wind velocity in m/sec	H_2O content in g per dm^2	Transpiration rate in g per hr per dm^2	Excess of leaf temperature over air temperature
			1954				
6/13	34.5—35.2	32	2	2.1	1.64	0.84	+3.6 to +3.9
			Certain plants showing scald				
7/7	39.1—40.2	12	0	2.0	1.44	1.02	+3.9 to +4.4
			Many plants showing scald				
7/11	39.8—40.4	17	1	3.0	1.54	0.87	+3.7 to +4.3
			Certain plants showing scald				
			1955				
6/17	24.8—25.6	47	4	3.4	1.74	1.06	−0.2 to −0.4
6/22	28.7—29.7	45	4	2.7	1.67	1.03	−0.3 to −0.7
7/19	22.1—22.4	44	3.5	2.1	1.65	1.03	−0.1 to −0.3
7/19	22.7—23.4	42	3.0	2.0	1.87	1.20	−0.3 to −0.6

TABLE 2

Temperature of the Leaves of Liutestsens 758 Wheat (at ordinary humidity) 1955 Experiment

Time of day (hour)	Temperature		Difference	Relative humidity in %	Time of day (hour)	Temperature		Difference	Relative humidity in %
	Leaf	Air				Leaf	Air		
	Sprouting June 10					Tillering June 27			
9-10 a.m.	29.7	29.9	−0.2	46	9—10	22.8	23.5	−0.7	51
12-1 p.m.	30.3	31.9	−1.6	32	12—13	26.7	27.7	−1.0	42
3-4 p.m.	31.1	32.0	−0.9	38	15—16	28.3	28.5	−0.2	44
5-6 p.m.	28.9	29.4	−0.5	54	17—18	25.4	25.6	−0.2	44

plant with a sufficient quantity of organic acids, to which they attribute a protective function. According to their opinion, this consists in the binding of ammonia which is formed in the plant at high temperatures. Introduction of organic acids into the plant by application to the roots brought about an increased heat resistance. Unfortunately, in this work no precise determinations of heat resistance were made, although several reliable methods of doing this are available in the literature [11, 15, 16].

Our experiments were carried out in 1954 in Valuevska at the P. A. Kostychev experimental improvement station, and in 1955 – at Shadrinsk at the collective experiment station, on the fields of the "Lenin Covenant" collective.

Plant growth in 1954 took place under very unfavorable conditions, especially in the first half of the growing period. Thus, for example, a total of 5.4 mm of rain fell in June. In this period there were nine periods of dry winds. There was about half a day when the maximum temperature exceeded 35° and many days when the relative humidity dropped below 20%. On certain days it went as low as 11 and even 8%.

The following year, 1955, was distinguished by marked fluctuations in the weather during the latter part of May and the first half of June. This period corresponded with the stages of sprouting and commencement of tillering of wheat and was strongly reflected in plant yields. On the fields of the Shadrinsk collective experiment station during this time the high air temperatures resulted in an intense heating of the soil. The temperature of

the superficial layer of soil rose during the day to 58.5°, and at night fell to 4°. Such a radical change in temperature had no preceptible effect on the physiological peculiarities of the plants.

Plants used in this investigation were: Liutestsens 758 wheat, which comprises more than half of the crops in the Kurgansk district, corn var. VIR-42, and Zhdanov 8281 sunflower. In certain cases the weedy plants, field sow-thistle – Sonchus arvensis – and spurge, Euphorbia virgata, were used.

Leaf temperature was measured with a microelectrothermometer, which is based on the principle of change of resistance of an electrical conductor as a function of temperature. To measure leaf temperature it was sufficient to place the needle to the measuring device in contact with the object whose temperature was to be measured. The accuracy of measurement was 0.1°. In addition the atmospheric conditions to which the top of the plant was exposed were precisely evaluated (air temperature, wind velocity and cloudiness). In many experiments the rate of transpiration was determined by the weighing method, and the water content was also measured.

In Table 1 are assembled data from field experiments which show that in 1954 the leaf temperature of sunflower was, in the majority of cases, 3.3-4.4° higher than the temperature of the surrounding air. Many plants showed scald symptoms. A somewhat lower water content of the leaves, a low transpiration rate, and a very low soil moisture content were also observed.*

In the 1955 experiments, the leaf temperature was 0.1-0.7° lower than the temperature of the surrounding air. The data on water content of leaves and on transpiration for the two years differ only slightly.** Nevertheless, marked differences in atmospheric and soil factors were observed (temperature and relative humidity of the air, cloudiness, soil moisture).

The temperature of leaves varies markedly with varying atmospheric conditions. Overheating of leaves is the result of comparatively low transpiration activity under conditions of marked soil-atmosphere dryness. The transpiration rate of 1.03-1.24 g per hour per dm^2 observed in the 1955 experiments (under fairly favorable atmospheric conditions) was quite sufficient to cool the leaves. The transpiration rate observed in 1954, 0.86-1.02 g per hour per dm^2, was clearly inadequate to protect leaves from overheating under such unfavorable soil-climatic conditions.

Observations of the temperature of leaves of Liutestsens 758 wheat, which were made four times during each of the sprouting and tillering stages, failed to show any increase in leaf temperature. It was always 0.2-1.6° lower than the temperature of the surrounding air (Table 2).

Overheating of leaves was also absent in the experiments of Novoselova [17] with varieties of spring wheat under conditions of soil-atmosphere dryness which were carried out in 1954 at the Valuevska experiment station. Their temperature was always lower than that of the surrounding air.

In contrast to wheat, the temperature of leaves of corn var. VIR-42 exceeded that of the surrounding air, sometimes by 1.0-3.1° (Table 3).

Corn leaves were more strongly heated in the middle of the day (3.1°), while in the morning and evening hours the leaf temperature exceeded the air temperature by only 0.2-0.4°.

The situation was different with respect to sunflower var. Zhdanov 8281, the temperature of whose leaves was 0.8-1.6° lower than that of the surrounding air in the middle of the day (Table 4).

In order to clarify the role of transpiration in keeping the leaves cool, experiments were designed in which, by various methods, transpiration was in greater or less degree suppressed. Plants were isolated in cellophane envelopes, leaf surfaces were smeared with vaseline, or the veins were cut (sunflower). In all experiments the transpiration rate and the water content, which was based on the same area of the leaf, were quantitatively evaluated along with the temperature.

The results were identical for all experiments. When transpiration is suppressed, leaf temperature always increases to a level 1.4-6.9° higher than that of the surrounding air. Prolonged maintenance of plants under

*In a number of cases the moisture content in the upper 40 cm of soil approached the wilting coefficient. In the 1955 experiments soil moisture content was 65-85% of the saturating amount.

**The low transpiration rate in such harsh atmospheric conditions is due to low soil moisture content.

TABLE 3

Temperature of Leaves of Sprouting Corn on June 14 (at ordinary humidity) 1955 Experiment

Time of day (hour)	Temperature		Difference	Relative humidity in %
	leaf	air		
9-10 a.m.	28.4	28.0	+0.4	46
12-1 p.m.	29.9	28.9	+1.0	41
3-4 p.m.	31.4	28.3	+3.1	37
5-6 p.m.	29.7	29.5	+0.2	37

TABLE 4

Temperature of Leaves of Sprouting Plants of Sunflower var. Zhdanov 8281 on June 12 (at ordinary humidity) 1955 Experiment

Time of day (hour)	Temperature		Difference	Relative humidity in %
	leaf	air		
9-10 a.m.	29.3	29.7	−0.4	34
12-1 p.m.	31.4	32.2	−0.8	37
3-4 p.m.	32.4	34.0	−1.6	39
5-6 p.m.	30.8	31.1	−0.3	41

TABLE 5

Dependence of the Temperature of Leaves of Sunflower var. Zhdanov 8281 and Corn var. VIR-42 on Water Content and Transpiration Rate

Plant	Air temp. in °C	Leaf temp. in °C	Difference between leaf temp. and air temp.	Overheating (excess of leaf temp. over the control air temp.)	Water content in g per dm^2	Transpiration rate in g per dm^2 per hour
July 18, 1955. Air temp. 26.1-26.5°. Cloudiness 4 units. Relative humidity 50%. Wind velocity 1.3-2.1 m/sec						
Sunflower in a cellophane envelope	29.1	34.2	+5.1	+6.9	1.98	0.56
Control (plants without cellophane)	27.3	26.8	−0.5	—	1.72	1.31
Sunflower with vaseline smeared over the leaf surfaces (for prevention of transpiration)	26.4	29.7	+3.3	+3.6	1.70	0.51
Control	26.1	25.8	−0.3	—	1.63	1.11
Sunflower with veins severed to decrease transpiration	26.5	27.9	+1.4	+1.4	1.26	0.32
Control	26.5	26.2	−0.3		1.51	1.33
Corn in a cellophane envelope	28.1	30.4	+2.3	+4.4	1.84	0.42
Control	26.0	27.2	+1.2		1.29	0.97
Corn with leaves smeared with vaseline	26.8	29.9	+3.1	+2.9	1.31	0.31
Control	27.0	28.4	+1.4		1.26	0.90
July 30, 1955. Air temperature 24.0-24.4°. Cloudiness 3 units. No wind						
Sunflower in a cellophane envelope	26.2	28.3	+2.1	+4.3	1.97	0.32
Control	24.0	27.3	+3.3		1.96	1.01
Sunflower with leaves smeared with vaseline	24.0	27.1	+3.1	+2.9	2.01	0.32
Control	24.2	24.0	−0.2		1.93	1.21
Sunflower with veins cut	23.4	26.8	+3.4	+2.4	1.11	0.56
Control	24.4	24.2	−0.2		1.77	1.43
Corn in a cellophane envelope	25.8	27.9	+2.1	+4.1	1.37	0.42
Control	23.8	25.6	+1.8		1.32	0.99
Corn with leaves smeared with vaseline	25.0	26.9	+1.9	+3.6	1.31	0.31
Control	23.3	24.6	+1.3		1.27	0.87

Fig. 1. Scald on sunflower leaves.

cellophane caused irreversible heat injury which was manifested in a browning of the leaf or part of it (Fig. 1). We observed similar heat injury in sunflower leaves in field experiments of 1954 as well as in crops at the Valuevska experiment station after watering. In this connection it is important that unwatered plants showed no heat injury. Corn, var. VIR-42, the leaves of which can withstand high air temperatures, was highly resistant to scald. Even when corn plants were kept under cellophane for prolonged periods, they showed no symptoms of scald, which is evidently due to a high resistance to overheating.

Transpiration is not the only factor involved in cooling of plants, however. Water content of the leaf also has an effect on leaf temperature. According to the data of Kleshnin [18], the temperature of leaves of artificially illuminated plants was higher in those leaves containing a larger quantity of water.

In order to elucidate the influence of water content on leaf heating, we set up special experiments. Experimental plants used were: spurge (Euphorbia virgata), field sow-thistle (Sonchus arvensis) and Zhdanov 8281 sunflower (Helianthus annuus), which have, in the order of listing, a decreasing water content of the leaf per unit area. Observations of the sunflower were carried out on the cotyledons and the true leaves. In some cases, in order to increase the water content of sunflower leaves, plants were watered in the evening and then covered with heavy paper. In addition to temperature, water content per unit area and transpiration were measured, and atmospheric factors were evaluated (Table 5).

Analysis of the experimental data shows a dependence of leaf temperature on leaf water content, i.e., the more water the leaf contains, the lower the transpiration rate and the more pronounced the heating effects. It is known that the temperature of water increases markedly when the rate of evaporation is greatly lowered. For example, Nikolaev, Vdovenko and Pokhil [19], covering pure water with a film of kerosene, naphtha, and "Olenaphtha"* 3-5 mm thick (to prevent evaporation) and comparing the temperature of water without a film, found marked differences in temperature. The greatest difference, 19.3°, was found with water covered with a film of olenaphtha.

The overheating of leaves which we have noted (Table 6) is characteristic of plants with a higher water content per unit leaf area, i.e., with a certain degree of succulence. Such a relationship is not found in other plants. The leaves of corn, for example, having a somewhat lower content of water per unit area and in most cases a lower transpiration rate, are heated to a greater degree than leaves of sunflower. Still more severe overheating, 4-4.5°, was observed in leaves of the bramble growing in thickets (Rubus saxatilis), although the water content of the leaves is one and a half times less than that of sunflower leaves.

The study of the heat regime of cultivated plants under natural conditions is concerned with one of the aspects of the total problem of heat resistance. Another aspect is involved in the study of the modifications and interchanges of the colloidal-chemical properties of protoplasm which take place in the plant at high temperatures. In this connection it was of interest to follow the modifications of certain of these properties which are characteristic of heat resistance.

The temperature threshold of coagulation, viscosity, permeability, and degree of hydrophily of colloids of the protoplasm are characteristic for heat resistance.

Heat resistance was studied by determining the coagulation threshold in sections of upper epidermis, in which the temperature (over a 10-minute period) at which all cells died was measured [11]. Protoplasmic viscosity was determined by the plasmolytic method [20]. Permeability was determined by conductivity [20], and hydrophily by the method of Dumanskii [21].

Liutestsens 758 wheat plants and Zhdanov 8281 sunflower plants were dug up with a ball of soil to prevent injury to the root system and placed in glass containers 40-45 cm in diameter. After watering of the soil the

* Apparently a Russian trade name – Publisher's note.

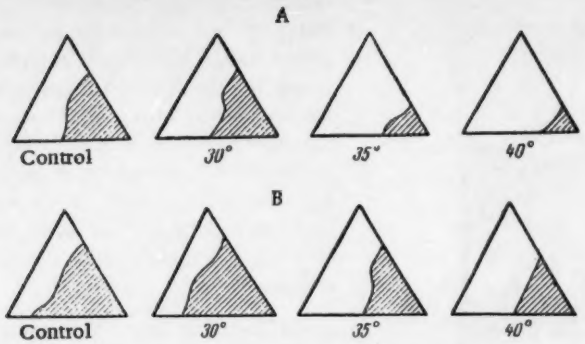

Fig. 2. Area of complete coagulation of hydrophilic colloids of leaves of Liutestsens 758 wheat at high temperatures. A) Sprouting stage; B) heading stage; shaded part – area of complete coagulation of hydrophilic colloids.

TABLE 6

Water Content and Temperature of Leaves of Sunflower var. Zhdanov 8281, Spurge and Field Sow Thistle

Plant	Date	Air temp. in °C	Relative humidity in %	Water content in g per dm^2	Transpiration rate in g per hr per dm^2	Leaf temp. in °C
Sunflower (true leaves)	6/27	29.1–29.6	38	1.57	1.12	−1.2
Sunflower (cotyledons)	6/27	29.7–31.2	38	4.84	0.38	+2.1 to +2.8
Sunflower (watered in the evening, and then covered with paper)	7/22	29.2–30.1	43	1.87	1.56	+0.6 to +0.8
Sunflower (control)	7/22	22.4–30.0	43	1.59	1.63	−1.3 to −1.4
Spurge	8/2	30.7–31.6	34	3.84	0.95	+2.6 to +3.1
Field sow thistle	8/2	30.9–31.4	34	3.21	1.24	+1.6 to +2.3
Sunflower	8/2	31.2–31.7	32	1.57	1.21	−0.6

TABLE 7

Permeability of Leaves of Liutestsens 758 Wheat at Various Temperatures (in ohms × 10^{-6})

Stage of development	Control	Temperature in the thermostat in °C		
		30	35	40
Sprouting	293	364	744	1721
Tillering	252	312	622	1052
Heading	264	292	492	1112

containers were lined at the side and covered with asbestos to decrease drying and heating of the soil. The plants were then placed in an illuminated thermostat and subjected for 2 hours to temperatures of 30, 35, 40 and 43°. Plants with uninjured root systems, i.e., those from the center, were analyzed.

TABLE 8

Heat Resistance and Viscosity of Protoplasm of Leaves of Liutestsens 758 Wheat

Treatment	Sprouting		Tillering	
	Heat resistance of cells	Protoplasmic viscosity	Heat resistance of cells	Protoplasmic viscosity
Control	48	14	50	24
Temperature				
30	50	26	52	32
35	48	36	52	34
40	47	40	49	46
43	46	46	48	50

One of the causes of the disturbance of the colloidal-chemical status of the protoplasm which is brought about by high temperature is the increase in permeability to all substances, especially to electrolytes. Experiments showed that the temperature at which an abrupt change in permeability, and consequently a marked disruption of the colloidal-chemical structure of the protoplasm of wheat takes place, is between 35 and 40° (Table 7).

The degree of heat resistance of a plant may also be evaluated in terms of the degree of hydrophily of the colloids; the correlation of these two factors has been repeatedly indicated in the literature [13-22]. A two-hour exposure of wheat plants to a temperature of 30° in an illuminated thermostat does not bring about any significant decrease in the area of complete coagulation of the hydrophilic colloids of the protoplasm during the sprouting stage, but in the heading stage, on the contrary, there even occurs a certain increase. Exposure to temperatures of 40 and 43° leads to a marked decrease in the area of complete coagulation during sprouting, which is directly related to a low heat resistance. A decrease in the area of complete coagulation is also found in the heading stage, but it is not as marked (Fig. 2).

With Liutestsens 758 wheat plants kept at 30° we found a certain (1-2°) increase in the coagulation threshold in the sprouting stage and also in the tillering stage (Table 8). The viscosity of the protoplasm was also increased under these conditions. More intense heating (at 40-43°) led to a marked lowering of the coagulation threshold. Viscosity continued to increase (Table 8).

The data we have obtained on hydrophily of colloids, protoplasmic viscosity and heat resistance clearly attest to the fact that hydrophilic viscosity of protoplasm under the influence of high temperature is lowered while the structural viscosity is increased. There is a decrease in heat resistance which parallels the decrease in hydrophilic viscosity. Our experiments showed that under the influence of high temperature the correlation between hydrophilic and structural viscosity of the protoplasm disappears.

SUMMARY

The temperature of leaves of various agricultural plants may vary greatly. The temperature of wheat leaves is in all instances lower than that of the air about them, whereas the temperature of sunflower leaves and those of corn differ greatly at midday, sunflower leaves exhibiting a temperature below that of air, while the temperature of corn leaves is somewhat higher than that of the air.

The temperature of plant leaves possessing a certain degree of succulence, i.e., possessing a considerable amount of water per unit of surface, as a rule is above that of the air about them (Sonchus, Euphorbia). For many other plants no regular correlation between moisture content and leaf temperature was observed.

Overheating of the sunflower leaf caused by enclosing the leaf in a cellophane envelope, cutting the veins of the leaf or applying vaseline to its surface, leads to heat injury. Corn, as a highly heat-resistant plant does not experience heat injury under these conditions.

When whole wheat plants of the Liutestsens 758 variety were enclosed in an illuminated thermostat at a high temperature, it was found that up to a certain point (30°C) the amount of hydrophilic colloids increased, and after 35°C sharply declined.

The coagulation threshold of protein protoplasm increases up to 30°C and after 35°C sharply declines. The viscosity of protoplasm continues to increase up to a temperature as high as 43°C.

Growing plants become heat resistant if exposed to a temperature of 30°C for a short period of time.

The decrease of heat resistance of a plant is due to decline of hydrophilic viscosity of the protoplasm and hydrophilic colloids, whereas the structural viscosity simultaneously rises.

In conclusion I wish to express my deep gratitude to Professor P. A. Genkel, who directed the work.

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SOME PHYSIOLOGICAL PECULIARITIES OF HARD SPRING WHEAT IN RELATION TO METHODS OF TILLAGE OF FALLOW SOIL

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The party and the administration have had in view a program of rapid improvement of the total national economy of our country. In the area of agriculture the important objective is the improvement of the yield of all cultivated crops.

For the attainment of this objective agronomic science has been obliged to consider and scientifically test those agronomic methods which would guarantee the best conditions for plant growth and would give high and resistant yields of agricultural crops.

T. S. Maltsev, creatively extending the study of V. R. Vil'iams, proposed a new system of agriculture which increases soil fertility and radically changes the view of annual plants. The methods proposed were examined in long-term experiments in terms of yields under actual conditions on the "Lenin Covenant" collective in the Shadrinsk district of the Kurgansk region, and also on other collectives of this district.

The All-Union conference of scientific and technological agriculturists which took place under the auspices of the Central Committee of the Communist Party of the Soviet Union in August of 1954 at the "Lenin Covenant" collective acknowledged the great scientific and practical significance of the methods proposed by T. S. Mal'tsev [1].

At the conference it was resolved to organize field tests in a study of these methods in the principal grain-growing regions of the USSR, among which is the Stalingrad region. It is obvious that a mechanical adoption in the Stalingrad region of the methods intended by T. S. Mal'tsev for the soil-climatic conditions of the trans-Urals is untenable. T. S. Mal'tsev himself has pointed out the necessity of a creative adaptation of these methods to new conditions.

Experiments at the "Lenin Covenant" collective farm were carried out chiefly on leached chernozems with a high water table (from 1 to 4.5 m). Soils were rich in humus and easily available mineral elements.

The light chestnut soils of the Stalingrad region contain an inadequate amount of minerals and humus and have a very low water table.

The inadequate precipitation in this region and also the Kurgansk region, and frequently its complete absence in an annual cycle, together with recurrent dry winds, induces harsh soil and atmospheric drought conditions, even without taking into account the deep water table; the significance of water, which is the chief limiting factor in yield production, is thus greater on the semi-arid steppes of the Stalingrad district.

In 1954-1955 the agricultural division of the Stalingrad region agricultural experiment station set up an experiment in which soil which had lain fallow for 20 years was given three basic treatments: 1) plowing with a plow fitted with a complete plowshare to a depth of 25-27 cm, with a further deepening of 10-15 cm; 2) plowing with a plow without a moldboard to a depth of 35-40 cm; 3) plowing with a shallow-disk plow in two perpendicular directions at a depth of 5-7 cm. These treatments were carried out in the fall of 1954.

The plowing was done with a P-5-35-P plow. With a plow fitted with a complete plowshare, the different soil horizons of varying fertility were mixed; the upper fertile layer was plowed under. For the second soil treatment, the plow P-50-P, with removable moldboard, was used. Under these conditions the soil horizons remained virtually intact, according to Mal'tsev; the most fertile surface layer is undisturbed. Plowing with a shallow disk plow loosens only the upper fertile layer to a depth of 5-7 cm.

The different methods of soil preparation results in varying aeration and moisture conditions, and biological processes in the soil – accumulation of mineral elements, microbiological processes, structure formation, etc. – proceed at different rates. In connection with this, physiological processes in plants, as the response of the plants themselves to changing environmental conditions, will also proceed at different rates.

A knowledge of the changes in the physiology of plants which occur with various soil treatments is necessary for a correct assessment of these treatments and their improvement. To obtain such information, a study of certain aspects of the physiology of hard spring wheat in relation to tillage methods was undertaken.

In the spring of 1955 (March 27) the soil on each experimental plot was harrowed to give a protective mulching. Soils receiving the third treatment were tilled twice with a shallow-disk plow to a depth of 5-7 cm and harrowed once. All soils were disked in a direction perpendicular to the previously plowed furrows just before sowing. The third tillage treatment comprised a total of five separate diskings.

Hard spring wheat var. Melanopus 69 was sown on April 13, 1955 on the basis of 60 kg/hectare; the seed was sown in a mixture with granulated superphosphate (50 kg/hectare). For 24 hours before sowing, the seeds were treated with a fungicide. The sowing was performed with a seed drill SB-48 drawn by a tractor. Shoots were first noted on April 29 (on May 1 on disked soils of treatment three).

The year 1955 was not typical of the Stalingrad region with respect to climatic conditions – there was a greater amount of precipitation than usual during the growing period, and no dry winds.

In connection with the problems at hand the following observations were made: increase in plant dry weight and leaf area during the growing period was determined for plant samples from five sites on each replicate plot. The samples consisted of 20-60 plants. The transpiration rate was determined in the field in the hottest hours of the day (12-1 pm) by the weighing method [2]. The carbon content of dried samples was determined by the method of Tiurin and Lukashik [3]. A single determination of the active absorptive surface of the roots according to the soil horizon was made by the method of Kolosov [4, 5]. Measurements of soil moisture content were also made. All such measurements were made on light chestnut soils poor in minerals.

The data on the increase in assimilation apparatus, the leaves, during the growing period afford an index of the physiological peculiarities of spring wheat in relation to soil tillage methods (Table 1).

These data show that on June 7 (beginning of heading) the number of leaves on plants grown on unturned soil (treatment 2) was 7% less than that on plants grown on soil which was turned under (treatment 1), and their weight and area were 30% lower, i.e., in these plants there were not only fewer leaves but the leaves were smaller. The difference in leaf size was even more evident on June 20 and July 5, when the number of leaves of treatment 2 was markedly greater (12%) while their weight and area were almost identical with those of the leaves of treatment 1 (a difference of 2-3% – within the limits of error). It is evident that the preceding tillage treatment in which the soil is not vertically mixed to any great extent (treatment 2) yields plants with leaves of a more xeromorphic type – they have a small surface area.

TABLE 1

Increase in Dry Mass and Area of Living Leaves of Hard Spring Wheat in the Different Experimental Groups (average of 100 plants)

Element measured	5/20	6/7	6/20	7/5
Plowed to a depth of 25-27 cm with a further deepening of 10-15 cm				
Number	900	1590	627	158
Dry weight (in g)	93.75	258.28	84.85	12.36
Area (in cm^2)	6827	21832	9285	1967
Plowed to a depth of 35-40 cm without turning under the soil				
Number	733	1481	747	188
Dry weight (in g)	76.90	180.74	82.26	12.14
Area (in cm^2)	5600	15278	9001	1933
Disked to a depth of 5-7 cm				
Number	660	1478	435	—
Dry weight (in g)	49.45	142.96	41.35	—
Area (in cm^2)	3600	12085	2524	—

TABLE 2

Carbon Content of Leaves of Hard Spring Wheat in the Different Experimental Groups (in % of dry material)

Soil treatment	5/20	6/7	6/20	7/5	
	Living leaves			Living	Withered
Plowed to a depth of 25-27 cm with a further deepening of 10-15 cm	39.38	39.68	40.80	40.43	37.20
Plowed to a depth of 35-40 cm without turning under the soil	39.80	39.68	39.98	39.60	37.73
Disked to a depth of 5-7 cm	39.80	39.68	40.65	—	37.35

TABLE 3

Dry Weight of Living and Withered Leaves of Hard Spring Wheat in the Different Experimental Groups (average of 100 plants)

Soil treatment	Total weight of leaves	Living		Withered	
		in g	in %	in g	in %
Observation of June 2					
Plowed to a depth of 25-27 cm with a further deepening of 10-15 cm	56.30	37.69	66.94	18.61	33.06
Plowed to a depth of 35-40 cm without turning under the soil	50.73	39.90	78.65	10.83	21.35
Disked to a depth of 5-7 cm	30.01	19.91	66.34	10.10	33.66
Observation of July 5					
Plowed to a depth of 25-27 cm with a further deepening of 10-15 cm	31.23	10.99	35.19	20.24	64.81
Plowed to a depth of 35-40 cm without turning under the soil	22.09	10.43	47.22	11.66	52.78
Disked to a depth of 5-7 cm	14.43	—	—	14.43	100.0

TABLE 4

Productiveness of the Leaves of Hard Spring Wheat (in g dry material per 1 cm^2 area of the leaves)

Soil treatment	5/20	6/7	6/20
Plowed to a depth of 25-27 cm with a further deepening of 10-15 cm	0.40	0.79	3.23
Plowed to a depth of 35-40 cm without turning under the soil	0.42	0.86	3.28
Disked to a depth of 5-7 cm	0.43	0.91	6.59

Even on May 20 the condition of plants grown on disked soil (treatment 3) was markedly poor in comparison with that of plants grown on soils given treatment 2. While the number of leaves was 10% less, their weight and area were 35% less. On June 7 the number of leaves was the same while their weight and area were 20% lower; on July 5, in the milk stage, all the leaves had withered, although the grain was not yet ripe.

The rate of photosynthesis may be evaluated by the carbon content of the leaves (Table 2).

These data show that at the very beginning, phososynthesis is most rapid in plants growing on unturned soil and disked soil; at the beginning of the heading stage it proceeds at the same rate under all conditions, after which it is more rapid where the soil has been turned over. In this connection the higher carbon content of withered leaves of plants on disked and unturned soil in comparison with that of leaves of plants on turned soil should be noted (observation of July 5). This is apparently due to the conditions of the plants themselves (Table 3) and the weather conditions in the period between June 20 and July 5.

Table 3 shows that at both times of measurement leaves of plants growing on unturned soil assimilate more vigorously because of better conditions. This would indicate a greater vitality of the plants themselves and a greater crop production.

From June 20 to July 5 there was no precipitation; the air temperature rose to 33° with an average daily temperature of 24.8°, and the temperature of the soil at a depth of 10 cm reached 31.5°, and at a depth of 20 cm – 32.5°; prevailing winds were from the south at 10 m per second.* These climatic conditions accounted for the rapid withering of the leaves. The greater carbon content of withered leaves of plants grown on unturned soil as compared with that of leaves of plants grown on soil which had been turned over is due to the fact that the flow of photosynthetic assimilates from the leaves was arrested at the time they dried up.

The photosynthetic work performed by the leaves is expressed by the weight of accumulated organic material in the plant per unit area of the leaves (Table 4).

It can be seen in Table 5 that the productivity of leaves of plants grown on unturned soil is greater than with plants grown on soil which has been turned over. Plants grown on disked soil are markedly different in this respect. We did not make any measurements for July 5, since at this time the majority of leaves had withered, and plants on disked soil had no living leaves at all.

The high index of productivity of leaves of plants grown on disked soil is related to conditions which suppressed plant growth and which were manifested in the decreased leaf surface (see Table 1) in comparison with plants of the other groups, as well as in the low dry weights and low average daily growth increments throughout the entire period (Table 5).

TABLE 5

Dry Weight and Average Daily Growth Increment of Plants of Hard Spring Wheat (in g)

Soil treatment	Dry weight of 100 plants				Average daily growth increment			
	5/20	6/7	6/20	7/5	4/29 - 5/20	5/21- 6/7	6/8- 6/20	6/21- 7/5
Plowed to a depth of 25-27 cm with a further deepening of 10-15 cm	27.45	172.96	299.55	348.95	1.25	8.08	9.74	3.36
Plowed to a depth of 35-40 cm without turning under the soil	23.51	131.74	295.52	321.24	1.07	6.01	12.60	1.71
Disked to a depth of 5-7 cm	15.39	109.59	166.42	134.11	0.77	5.23	4.37	—

TABLE 6

Distribution of the Root System of Hard Spring Wheat (in %)

Soil treatment	Weight of roots of a horizon, in cm			Area of the activity absorbing surface of the roots in a horizon, cm		
	0–10	10–27	27–40	0–10	10–27	27–40
Plowed to a depth of 25-27 cm with a further deepening of 10-15 cm	49.8	33.1	17.1	30.7	34.9	34.4
Plowed to a depth of 35-40 cm without turning under the soil	35.0	39.3	24.7	26.4	43.8	29.7
Disked to a depth of 5-7 cm	52.7	22.5	24.8	43.5	27.5	29.0

* Meters per second. Editor.

Table 5 shows that the dry weight of plants grown under conditions normal for them (on deep-plowed soil where the soil may or may not be turned) steadily increased, and the average daily growth increment during the heading-flowering stage was observed in plants grown on unturned soils. In plants grown on disked soil the absolute values for dry weight and average daily growth increment were lower than in either of the other groups. Moreover, because of the climatic conditions prevailing during the growth period there was a rapid desiccation of the leaves, some of which fell off with strong gusts of wind, so that the weight of the plants on July 5 had even decreased.

Such plant behavior can be explained in terms of the distribution of the root system (Table 6).

The distribution of the root system was determined on June 23, i.e., after cessation of heading. According to Nalivkin [6], root growth in wheat ceases at heading.

Table 6 shows that the main portion of the roots according to weight, which is composed chiefly of coarse roots, is located in the 0-27 cm horizon, and that more than 50% of the roots in disked soil are located in the 0-10 cm horizon.

The closest attention should be given to the distribution of the active absorptive surface of the root system involving the smallest, most active roots which supply the plant with water and minerals, i.e., those which are indispensable in crop production. The data show that the most uniform distribution of the active absorptive surface is found in plants grown on soil plowed to a depth of 25-27 cm and then further deepened.

In unturned soil, the main part of the active absorptive root surface is located in the 10-27 cm horizon (43.8%).*

On disked soils the main portion of the active surface (43.5%) is located in the uppermost horizon (0-10 cm). This impedes the withdrawal of water from the lower horizons, as the growth pattern of the plants (see Table 5), and also the final yield, shows. A root distribution of this kind would be useful to plants in the uptake of water from the small annual rainfall. The small amount of water which falls as rain (in May 10 mm, in June 46.8 mm and in July – 0), is evaporated from the soil surface faster than it is used by the plants under conditions of abundant sunlight and heat and prevailing south and southeast winds, and does not penetrate to a deeper soil level.

The data on transpiration rates in the hottest hours of the day also reflect physiological responses of plants to methods of soil tillage (Table 7).

TABLE 7

Transpiration Rate of Hard Spring Wheat
(in g water per hour per m^2 leaf surface)

Element measured	6/21	6/8	6/21	7/5
Plowed to a depth of 25-27 cm with a further deepening of 10-15 cm	72.11	378.35	204.2	147.4
Plowed to a depth of 35-40 cm without turning under the soil	470.06	346.80	262 4	196.0
Disked to a depth of 5-7 cm	276.51	562.11	211.5	—

It can be seen from Table 7 that the transpiration rate in the daylight hours is highest in plants grown on unturned soil, which is indicative of a greater plant vitality. The increase in transpiration during the hottest hours of the day, is, moreover, a protection against overheating.

* Genkel' and co-workers also obtained data on the position of the main portion of the active root surface in unturned soils at the "Lenin Covenant" collective in the Shadrinsk district [7], based upon T. S. Mal'tsev's experiments.

TABLE 8

Amount of Available Water in a Half-Meter Layer of Soil (in m^3/hectare)

Soil treatment	4/13	5/17	4/15	7/29
Plowed to a depth of 25-27 cm with a further deepening of 10-15 cm	1138	950	595	160
Plowed to a depth of 35-40 cm without turning under the soil	1349	870	776	189
Disked to a depth of 5-7 cm	763	464	297	—

TABLE 9

Yield, Field Transpiration Coefficient and Average Daily Consumption of Water of Hard Spring Wheat

Soil treatment	Grain yield in cwt/ hectare	Field transmission coef. in g water per g dry wt.	Average daily consumption of water during growing, in mm
Plowed to a depth of 25-27 cm with a further deepening of 10-15 cm	12.33	701.8	2.73
Plowed to a depth of 35-40 cm without turning under the soil	13.35	529.2	2.45
Disked to a depth of 5-7 cm	5.70	554.1	1.53

The high transpiration rate of plants grown on disked soil which was measured on June 8 – 562.11 g water per hour per m^2 leaf area – should be noted. This is related to the surface distribution of the roots which had utilized to the maximum extent the 46.8 mm of rain which had fallen from June 3rd to June 6th.

The observations described above showed that physiological processes are more active in plants grown on fallow soil which had been plowed without being turned over. This is due to the fact that more water is accumulated in a soil so treated (Table 8). The water content was assessed according to the method proposed by Mozharov [8].

The data of Table 8 show that more water is accumulated in unturned soil than in well-mixed soil. There was less water in disked soil at all times of measurement, and after the hervest (July 29) there was no available water at all.

The field transpiration coefficient of plants was determined by the method of Maksimov [9] and expressed as g water per g dry weight. (Table 9). The average daily consumption of water in mm/hectare [10] was determined at the same time.

Table 9 shows that when fallow soil was plowed to a depth of 35-40 cm in the spring, as suggested by T. S. Mal'tsev, there was a higher grain yield, a smaller transpiration coefficient, and a lower average daily consumption of water than when soil was plowed to a depth of 25-27 cm and further deepened 10-15 cm, i.e., there was a more economical expenditure of water.

The insignificant average daily consumption of water observed in wheat grown on disked soil was accompanied by an exceptionally low grain yield, attesting to the marked inhibition of the plants. Even this yield was obtained only because of the favorable weather conditions and the absence of dry winds during the 1955 growing period which are not typical of the semi-arid steppes of the Stalingrad region.

SUMMARY

Hard summer wheat grows better in the south of the Stalingrad region if fallow soil is plowed to a depth of 35-40 cm by the method of T. S. Mal'tsev than if plowed to a depth of 25-27 cm and further deepened by

10-15 cm. In particular, the number of leaves increases but their size is decreased whereas the total surface area remains constant and the wilting rate is less, which means greater vitality of the plant and larger crops; photosynthesis is higher; the distribution of the active absorptive surface of the root system is more favorable for the plant; transpiration at the hottest hours of the day is higher, thus protecting the plant from being overheated; at the same time the field transpiration coefficient and the average daily consumption of water decreases while the crop yield increases, i.e., water is consumed in a more economical manner. This is all accompanied by an accumulation of large quantities of useful water in the soil.

Disking of fallow soil, even under the favorable climatic conditions of 1955, suppressed plant growth. The observations discussed above and the low yield of grain confirms this conclusion.

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PRODUCTIVITY OF THE LEAF APPARATUS OF MAIZE UNDER IRRIGATION CONDITIONS

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The leaf is the site of the primary synthesis of organic substances [1]. Its activities, which are directly related to the accumulation of vegetable matter, play a leading role in crop production [2, 3]. Physiological processes taking place in leaf tissues are extremely labile. They are rapidly altered by the influence not only of environmental factors to which the aerial plant organs are exposed [4], but also by conditions of root nutrition [5].

The question of the interrelation of root nutrition and activity of the assimilatory apparatus has been given very little attention. We have set ourselves the problem of studying the relation of root nutrition of maize to the assimilatory activity of the leaves under both optimal and sub-optimal conditions of water and mineral element supply to the roots.

We have shown in previous investigations [6] that high productivity of maize under favorable conditions of water supply and mineral elements supply is to a marked degree determined by the physiological activity of the roots.

We report in this paper the results of further experiments, the purpose of which was to demonstrate changes in the assimilatory activity of maize leading to a greater plant productivity, which are directly related to root nutrition.

METHODS

The experimental conditions were the same as those described for a previous investigation [6]. The productivity of the leaf apparatus was determined by the method of Begishev [7]. Plant samples comprising 10-15 plants were selected every 10 days. For determination of leaf area, disks were cut out from an average sample. Knowing the weight and area of the disks and also the weight of a sample of leaves, it was possible to calculate the area of the leaves of a single plant.

The rate of flow of assimilates from the leaf to the ear was determined at flowering, swelling of the grain, and at the beginning of the milk stage according to the method proposed by Zholkevich [8]. The assimilatory activity was assessed according to the method of Zeller [9], and also according to the extent of uptake of radioactive carbon dioxide after exposure of the leaf to the labeled substance [8]. Reducing sugars and sucrose were determined in the leaves according to the micromethod of Bertrans [10]. An average sample for this determination consisted of 40-50 plants. We assessed plant growth by measurement of the height of 40 plants of each experimental group once every 5 days. Chlorophyll concentration was determined according to Godnev [11]. The average sample used was of the same size as for sugar determination.

The data on productivity of the leaf apparatus of maize obtained in 1955 and 1956 were in agreement with each other, and therefore they are not separately presented.

The following items, which define the water status of the cell, were determined every 5 days at 1 pm: suction tension by the method of Maksimov-Petinov [12], osmotic pressure according to Bardzher [13], free and bound water according to Marinchik [14], and total water content by drying to a constant weight of 105°.

EXPERIMENTAL DATA

Leaf productivity varied throughout the growing period (Fig. 1). At the beginning of growth it was low, by the end of the leaf formation period it had risen sharply, subsequently declining during the stage of tassel formation and during the flowering period it once more increased, reaching its highest level during the grain-swelling stage. A continuous supply of water to the aerial portion of the plant, as indicated by quantitative analysis of the sap [6] and hydration of the leaf tissue (Table 1), increases leaf productivity 1.5-2.5 times.

TABLE 1

Effect of Irrigation and Application of Fertilizer on Hydration of the Leaf Tissue of Maize

Stage of development	Total amount of water in the leaf in %	
	Unirrigated	Irrigated
Variety Dnepropetrovskaia		
Formation of leaves	80.7	81.7
Tassel formation	76.3	78.6
Flowering	75.2	76.3
Variety Slavgorodskaia 270		
Formation of leaves	83.5	85.0
Tassel formation	79.3	80.1
Flowering	72.0	79.4
Ear formation	72.8	75.8
Variety Odessa 10		
Formation of leaves	77.5	79.3
Tassel formation	76.4	82.0
Flowering	74.4	75.8

TABLE 2

Change in the Elements Contributing to the Productivity of the Photosynthetic Apparatus of Two Maize Varieties Under Irrigation Conditions

Variety	Stage of development	Rate of photosynthesis in mg CO_2 per hr/g dry wt.	Chlorophyll content in % of dry wt.	Total leaf area of one plant in m^2	Height of plant in cm
Slavgorodskaia 270	Formation of leaves	38.93	1.509	0.1	—
Odessa 10		24.36	1.340	0.6	—
Slavgorodskaia 270	Tassel formation	16.70	1.829	0.5	83
Odessa 10		14.95	1.200	1.7	191
Slavgorodskaia 270	Flowering	24.26	1.397	1.4	134
Odessa 10		16.96	1.509	1.7	259
Slavgorodskaia 270	Development of ear	21.59	1.367	0.5	151
Odessa 10		16.31	1.149	1.0	270
Slavgorodskaia 270	Milk stage	20.68	1.436	0.5	—
Odessa 10		6.62	1.223	0.6	—

It should be noted that the different maize varieties under irrigation conditions show an increase in productivity for different reasons. The data in Table 2 show that in the variety Slavgorodskaia 270 (early maturing, low growing) the increased assimilation can be complained mainly in terms of photosynthetic rates and chlorophyll content, while in the variety Odessa 10 (tall, late maturing) the high productivity is due primarily to a greater assimilatory surface and rapid growth.

The increase of dry matter in the individual plant (Fig. 2, curves 1 and 2) is determined to a significant extent by the amount and degree of activity of water in the plant cell.

The ontogenetic changes in suction tension and osmotic pressure (Fig. 3 and 4) in a downward direction reflect a hightened activity of the water-absorbing and water-conducting systems of irrigated maize plants; under these conditions the activity of water in the plant cell is increased [15]. A weak hydration of the leaf tissues is associated, on the contrary, with a marked increase in suction tension and osmotic pressure, indicating a

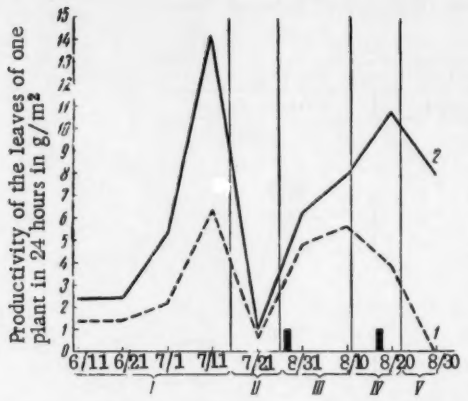

Fig. 1. Productivity of the leaf apparatus of maize var. Dnepropetrovskaia.
1) Unirrigated; 2) irrigated; I) formation of leaves; II) formation of tassels; III) flowering; IV) swelling of grain; V) milk stage.

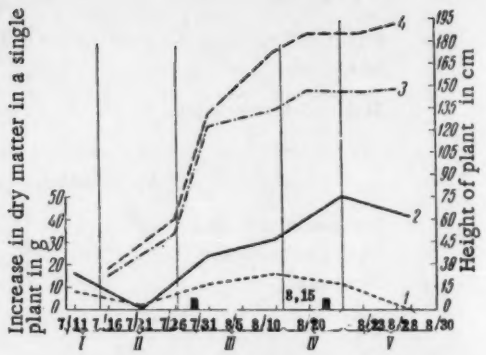

Fig. 2. Increase in dry matter of the aerial portion (curves 1 and 2) and growth (curves 3 and 4) of maize var. Dnepropetrovksaia.
1 and 3) Unirrigated; 2 and 4) irrigated; I) formation of leaves; II) formation of tassels; III) flowering; IV) development of the ear; V) milk stage.

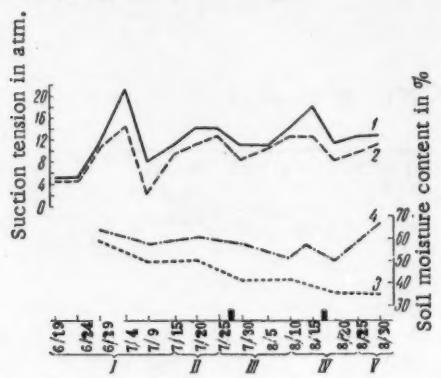

Fig. 3. Effect of irrigation on the dynamics of change in suction tension of maize var. Dnepropetrovskaia (curves 1 and 2) and on moisture content of the soil under the plants (curve 3) unirrigated, 4) irrigated.
1) Unirrigated; 2) irrigated; I) formation of leaves; II) formation of tassels; III) flowering; IV) swelling of the grain; V) milk-waxy stage.

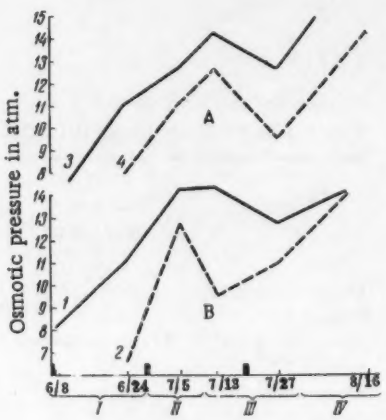

Fig. 4. Dynamics of change of osmotic pressure in leaf cells of maize var. Odessa 10 (A) and var. Slavgorodskaia 270 (B).
With irrigation – (curves 2 and 4) and without irrigation – (curves 1 and 3).
I) Formation of leaves; II) formation of tassels; III) flowering; IV) swelling of grain.

significant dislocation of the water status of the plant and leading ultimately to a substantial disruption of the synthetic function of the plant. Thus, as the water status of the cell is worsened, the dry weight and leaf area are adversely affected to a marked degree (Fig. 5).

In the unirrigated group, the assimilatory area steadily increases to the beginning of August. In the irrigated plants, however, it reaches its maximum value at the end of August, and subsequently remains at this high level during ear development and the milk stage.

Let us turn our attention to the intensive accumulation of organic material in the stage of flowering and ear development in the irrigated group (Fig. 2, curve 2). The active growth occurring during the flowering stage is suspended during grain development (Fig. 2, curves 3 and 4). Irrigation is associated with a change in

TABLE 3

Effect of Irrigation and Application of Fertilizer on the Ratio of Free to Bound Water in Leaves of Maize Variety Dnepropetrovskaia

Date samples were taken for analysis	Stage of development	Ratio of free to bound water	
		Unirrigated	Irrigated
June 14	Formation of leaves	1.43	0.96
June 20	Formation of leaves	0.58	1.34
July 30	Flowering of the male inflorescence	1.03	1.42
August 5	Flowering of the female inflorescence	0.70	1.30
August 20	Swelling of the ear	0.73	1.18
August 30	Milk stage	0.56	0.63

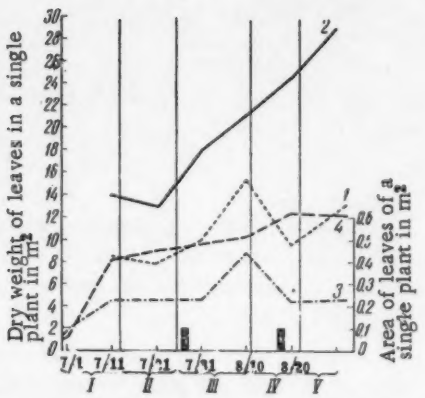

Fig. 5. Accumulation of dry matter in leaves (curves 1, 2) and dynamics of increase in leaf area (curves 3, 4) of maize var. Dnepropetrovskaia. 1 and 3) Unirrigated; 2 and 4) irrigated; I) formation of leaves; II) formation of tassels; III) flowering; IV) swelling of grain; V) milk stage.

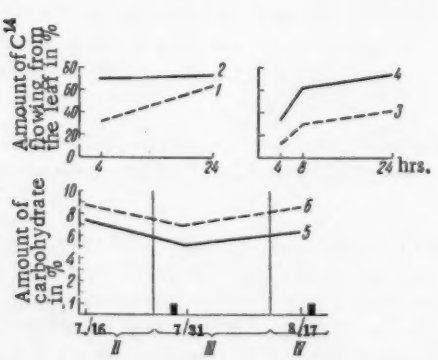

Fig. 7. Dynamics of outflow of assimilates from the leaf (curves 1 and 2) August 16; 3 and 4) August 21) and accumulation of sugars by leaves of maize var. Dnepropetrovskaia (curves 5 and 6).
1, 3, 6) Unirrigated; 2, 4, 5) irrigated; II) formation of tassels; III) flowering; IV) swelling of grain.

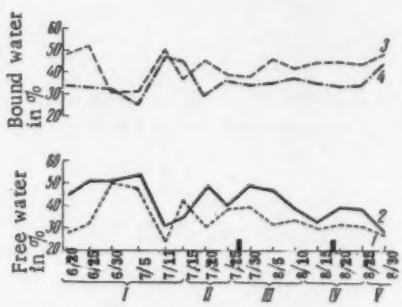

Fig. 6. Change in free water content (curves 1 and 2) and bound water content (curves 3, 4) in leaves of maize var. Dnepropetrovskaia under irrigation conditions.
1 and 3) Unirrigated; 2 and 4) irrigated; I) formation of leaves; II) formation of tassels; III) flowering; IV) swelling of grain; V) milky-waxy stage.

the ratio of free to bound water in the leaf tissues (Table 3), with free water becomming relatively more abundant (Fig. 6, curves 1 and 2); this ratio determines the rate of such important physiological processes as photosynthesis and assimilate flow (Fig. 7, curves 1-4; Table 4).

The curves for free water in both groups of plants are diametrically opposed to those expressing the dynamics of change of bound water during plant ontogenesis (Fig. 6). At the end of the growing period, when synthetic processes have ceased, the free water content has sharply declined while the bound water level is somewhat higher than formerly.

The amount and intensity of photosynthesis and assimilate flow are related to the physiological activity of the root system, which provides an increased amount of water and mineral elements to the plant during flowering [6]. A weak hydration of the leaf tissues is the cause of a decreased

TABLE 4

The Movement of Radioactive Carbon from the Leaf to the Ear in Irrigated and Unirrigated Plants

Treatment	Date of experiment and stage of plant development	C^{14} content in counts/min/g dry wt.				
		Leaf			Ear	
		Immediately after exposure to C^{14}	after 4 hrs	after 8 hrs	after 24 hrs	after 24 hrs
Unirrigated	Aug. 16 – flowering	110	80	—	40	60
Irrigated		4200	1260	—	1080	170
Unirrigated	Aug. 21 – swelling of grain	830	680	570	490	30
Irrigated		1230	800	480	330	90
Unirrigated	Aug. 28 – swelling of grain	880	500	310	200	—
Irrigated		1360	1150	770	160	—
Unirrigated	Sept. 5 – milk stage	3150	2180	1100	970	160
Irrigated		4030	1400	1000	660	380
Unirrigated	Sept. 10 – milk stage	2700	1900	—	1840	40
Irrigated		3250	1290	—	580	100

outflow of assimilates (Fig. 7, curves 1 and 3), the result of which is that the carbon content of leaves of unirrigated plants increases during the flowering and grain-ripening stages (Fig. 7, curve 6).

DISCUSSION

Katunski [16] has already speculated on the existence of critical periods in plant nutrition, i.e., periods of increased requirements of the plant for carbon. This requirement is closely related to conditions of root nutrition [17], which exerts an influence on the whole complex of physiological processes of the leaf, in which are included all the component parts of the photosynthetic process.

Our investigations support the hypothesis of critical periods not only with respect to carbon nutrition but also with respect to root nutrition. Of the greatest interest is the stage of flowering and grain development, when there is a marked alteration in many physiological processes involved in crop production: the rate of photosynthesis and assimilate flow from leaves is increased, the rates of increase of leaf area and dry matter of the aerial portions of the plant area altered, the behavior of many enzyme systems changes, etc.

In previous experiments we observed a heightened transport of water and minerals from the roots to the developing reproductive organs of maize [6]. As a result there is an improvement of the water status of the cells which, as is known, controls the rate of physiological processes in the leaf tissues [18]. During formation of inflorescences and flowering the amount of free water increases markedly (Fig. 6), and the relative amounts of the two forms of water become more favorable for growth (Table 3); the suction tension and the osmotic pressure are decreased, especially at flowering (Fig. 3 and 4). The optimal water and mineral status of the plant in its turn brings about a vigorous development of the aerial portion of the plant. The plant is thus conditioned to an increase in productivity [19].

Thus, as a result of the continuous increase in leaf area and dry matter under irrigation conditions [20, 21], the productive activity of the leaf apparatus of the tall, late-maturing varieties (Odessa 10, Dnepropetrovskaia) increases [4, 5].

The dwarf, early maturing varieties (for example, Slavgorodskaia 270) become more productive under irrigation conditions because of an increased rate of photosynthesis, and also to a certain extent because of an increase in the chlorophyll-protein component. The maximum productivity of the leaf corresponds with the period of development of the reproductive organs. As a result of vigorous development of these organs, transport of assimilates from the leaf is intensified and photosynthesis increases (Table 4). Leaves of irrigated plants are freed of assimilates more rapidly and more completely [8, 22], as is manifested by the lower carbon content in their tissues.

The root system of unirrigated plants is extremely inefficient, supplying to the leaves an insignificant quantity of water and minerals, [6]. Hydration of leaves of unirrigated plants is depressed [25]. The level of free water throughout the growing period is lower. The increase in bound water, especially in the second half of the growing period, under conditions of water deficiency occurs mainly in the osmotically bound fraction [23, 24]. Osmotic pressure and suction tension are increased. As a result of these changes in the water status the physiological activity of unirrigated plants is depressed. The development of reproductive organs and growth processes are slowed up [25, 26], the outflow of assimilates is depressed, carbohydrates accumulate in the leaf, and photosynthesis is depressed (Table 2).

Thus, an uninterrupted supply of water and minerals to the plant leads to substantial changes in the productivity of the leaf apparatus. In the most important and critical period of formation and development of the reproductive organs the leaf of irrigated maize works extremely efficiently: there is an uninterrupted vigorous increase in dry matter together with a normal development of assimilating tissue. At this time the leaves synthesize maximal quantities of organic materials per unit leaf area, and there is a heightened requirement for assimilates for the growth and development of the reproductive organs. The leaves rapidly lose their nutritive materials, thus creating favorable conditions for still more intensive synthesis which guarantees a high yield.

SUMMARY

The increase of dry weight and leaf surface, growth processes and also the productivity of the assimilatory apparatus are strongly dependent on the activity of the root system. Upon irrigation of maize the flow of substances and photosynthetic activity increases whereas the quantity of carbohydrates in the leaf decreases. The cells of the leaf tissues do not experience water deficiency duringthe day or throughout the vegetative period. The amount of free water was found to increase, the ratio of free to bound water also increased and the osmotic pressure and suction pressure decreased. These regularities are reversed under conditions of water or mineral nutrition deficiency.

During the flowering period and period of grain growth the requirements of maize in carbon and mineral nutrition are higher. If these requirements are met, many physiological processes become more active (e. g., photosynthesis, flow of substances, growth etc.) and as a result the productivity of the plant becomes higher. Accumulation of organic substances under irrigation conditions varies physiologically depending on the variety of maize. The productivity of the Slovgorodskaia 270 (early maturing) variety increases chiefly as a result of intensification of photosynthesis and increase in the amount of chlorophyll whereas productivity of the Odessa 10 (late maturing) variety is mainly due to intensification of growth processes and increase of the leaf surface.

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THE PROLONGED ACTION OF LIGHT OF DIFFERENT SPECTRAL COMPOSITION ON PLANTS

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We may assume that it has been firmly established, at least for green plants, that short-wave radiations during short-time action have a positive effect on the synthesis of nitrogen-containing organic substances. The reaction is first-order, independent of the length of exposure, of the systematic position of the object investigated, and of its physiological state [1-5]. According to some data, activation of this process begins from the first stages of nitrogen assimilation – from the process of nitrate reduction [6, 7]. The more rapid synthesis of nitrogenous substances is accompanied by a decrease in the amount of free carbohydrates [3].

As could be expected, much less clear results as to the effect of the quality of light on the accumulation of nitrogenous substances and carbohydrates were obtained for plants which were grown wholy in light of different spectral composition, when the processes of formation and accumulation of substances were complicated by the processes of growth and development. However, under these conditions, too, the favorable action of short-wave radiation on protein synthesis was noted [8-10]. Sometimes this reaction is very weakly expressed [9]. Insofar as carbohydrate accumulation is concerned, some investigators [8-11] found a smaller accumulation in plants grown in short-wave radiation as compared with those in long-wave radiation. Others [12] did not succeed in noting these differences.

One should bear in mind that in all works cited, the data on accumulation of organic substances in plants at various parts of the spectrum were not the same: accumulation was lower, and sometimes very considerably lower, in the short-wave radiation. The cause of this phenomenon is in the method of equalizing the light intensity – the light, in the best case, was equalized in intensity of the incident radiation. Because of this, less favorable conditions for photosynthesis, and consequently for accumulation of organic substances and carbohydrates, were created in the short-wave radiation. In such a case, it is difficult to refer changes in accumulation of these substances only to specific action of radiation of various wave lengths. Only in the experiments of Kleshnin, Osipova, and Timofeeva [9], who worked with several intensities of red and blue light, may the effect of the spectral composition of light on the metabolism of the substances be compared both in equality of light with relation to energy content, and the amount of incident quanta, and finally to the same accumulation of organic mass. However, these experiments were carried out at a very small total light intensity (up to 30×10^3 erg/cm^2/sec) and, as was pointed out earlier, no differences were obtained in carbohydrates, but the favorable effect of short-wave radiation on photosynthesis was weakly expressed.

Thus, we may consider that the existing data concerning the effect of the spectral composition of light on the nitrogen and carbohydrate metabolism of plants during extensive growth are exploratory in nature, and that the question needs further investigation.

According to the concepts developed by Nichiporovich [13], the nature and interrelationship of organic substances formed during photosynthesis may, to a considerable degree, determine the general direction of metabolism in plants, and in the long run affect the quality at least of the biological yields of plants. Because of this, it seemed to us interesting to follow to what degree and in what conditions, in case of the extensive growth of plants, should appear the reaction relating to the effect of the spectral composition of light on plant metabolism which had been established during short-term experiments.

Experimental Object and Methods

In connection with the problems of the investigation, we determined several biochemical indicators in 25-day-old bean plants (Phaseolus vulgaris), which were grown under conditions of illumination by light of various spectral compositions. In order to avoid the photoperiodic effect of the light of various qualities on plants [14], we chose for the experiment the neutral bean variety, "Triumph." The conditions of nitrogenous nutrition were

changed in the experiment, as it was assumed that the action of the spectral light composition on metabolism may depend upon the provision of plants with nitrogen. The beans were grown in sand on Hellriegel's mixture with nitrogen deficiency (1/4 normal) and a full level of the rest of the elements. Thirty-six hours before the termination of the experiment, half of the plants from each treatment were given nitrogen as calcium nitrate up to the full level of Helriegel's mixture. Thus, we analyzed plants which were grown all the time with nitrogen deficiency, as well as plants which received an additional dose of nitrogen. One experiment was set up under conditions of soil culture, without changing nitrogen nutrition, with a high level of nitrogen during the whole period of growth.

The plants were continuously illuminated. The lamps of "red" light (CdB_2O_5 phosphor) and "blue" light ($MgWo_4$ phosphor), 30 watt in intensity, were placed over the plants as a grill. As is known, these sources are not monochromatic. The spectral characteristics of the lamps used are given in Kleshnin's book, The Plant and Light [15]. The temperature at plant level under both sources of illumination was 26-27°.

The light in the experiments was equalized according to the number of incident quanta. The intensity of the light flux was measured by a Bell phytoactinometer [16] at the level of the first and second whorls of true leaves (from below) and was 50×10^3 erg/cm^2/sec for the red light and 80×10^3 erg/cm^2/sec for the blue light.

Before supplemental feeding of plants with nitrogen, their height, wet and dry weight, thickness of palisade and spongy parenchyma, leaf thickness, and chloroplast length were determined. For both treatments of nitrogen nutrition, the total chlorophyll content was determined with a Kenig-Marten's spectrophotometer. In the case of biochemical determinations, the material (the second and third whorl of true leaves) was fixed by dry heat at 105°, after which it was dried at 50°. The carbohydrate content in the dried and ground material was determined by the method of Bertran, according to the shortened procedure of Kizel and total and protein nitrogen (protein precipitation according to Barnshtein-Shtutfer) [17] and composition of free amino acids by the method of paper chromatography. Also, the amino acid composition of the chloroplast protein hydrolyzate was determined. The chloroplasts were isolated after preliminary grinding of leaves in cold 0.5 M sucrose solution in a meat grinder. Precipitation of chloroplasts from the suspension was carried out in a laboratory centrifuge at a speed of 10,000 rpm. The isolated chloroplasts were hydrolyzed with 20% HCl at 100° for 24 hours, after which the amino acid composition of the protein hydrolyzate was determined. For analysis of free amino acids, as well as of chloroplast protein amino acid, chromatographic paper "Leningradskaia" No. 2 was used. The solvent was butanol-acetic acid-water (4 : 1 : 5). Identification of the separate amino acids was carried out with the aid of ninhydrin, as well as of isatin, according to the method proposed by Boiarkin [18].

EXPERIMENTAL RESULTS

Bean sprouts appeared simultaneously in both red and blue light. At the time of analysis, the plants of both treatments were forming flower buds and flowering had begun. There was no noticeable difference in the rate of these processes for red and blue light. However, from the first day of sprouting the plants of both treatments began to differ in their appearance, particularly through differences in the rate of growth. In blue light, the plants were low and squat; in red light, they were elongated, with symptoms of etiolation. The formative effect of light on plants, observed by us, has been described in the literature [19]. The data on the wet and dry weight of plants are given in Table 1.

TABLE 1

Wet and Dry Weight of Plants Grown in Red and Blue Light

Light in experiment	Soil culture				Sand culture			
	wet weight		dry weight		wet weight		dry weight	
	weight in g	% of red to blue	weight in g	% of red to blue	weight in g	% of red to blue	weight in g	% of red to blue
Red	5,1	124	0,61	107	4.5	121	0.52	108
Blue	4,1	100	0,57	100	3.7	100	0.48	100

From the data given in Table 1, it can be seen that the weight of plants, in both red and blue light, is greater in soil culture than in sand culture. One may suppose that the lesser weight of plants in sand culture was conditioned by nitrogen deficiency in nutrient mixture. It should be noted that, independent of the condition of nitrogen supply, plants illuminated with red light were somewhat heavier in wet weight than those illuminated in blue light. The dry weight of both treatments differed insignificantly, from which it follows that the hydration of tissues was higher in red light than in blue light.

Thus, in our experiments, in the interrelationship of incident energy of red and blue light, equalized according to quanta, a practically similar accumulation of dry mass was ensured. Similar results were obtained earlier by Stolwijk [20].

We consider a similar rate of accumulation of organic substance in both parts of the spectrum an important condition for carrying out biochemical analysis, since in this case possible shifts in metabolism may be explained, not by a different rate of accumulation of organic mass, but by a specific action of different wavelengths.

TABLE 2

Anatomical Characteristics of Leaves Illuminated by Red and Blue Light
(indicator value is given in microns)

Light in experiment	Sand culture (1/4 normal nitrogen)				Soil culture			
	leaf thickness	Palisade parenchyma	Spongy parenchyma	Length of the chloroplasts	leaf thickness	Palisade parenchyma	Spongy parenchyma	Length of the chloroplasts
Red	33	11.5	16.5	1.0	34.8	15.4	15.0	2.0
Blue	43	18.0	14.5	2.2	37.6	15.6	16.0	3.5

In Table 2 are given some indicators of anatomical characteristics of plants illuminated by red and blue light. In the case of nitrogen deficiency in the nutrient medium, significant differences in leaf thickness favoring blue light were obtained through different lengths of palisade parenchyma cells in red and blue light. In soil culture, these differences were equalized.

More stable indicators were obtained in the size of chloroplasts. A somewhat larger chloroplast size was found in the blue light both for sand and for soil culture.

In Table 3 are summarized the data on content of nitrogenous compounds in leaves illuminated by red and blue light. First of all, one should note a considerably greater content of total, as well as of protein, nitrogen in the leaves under blue light as compared with red light, during nitrogen starvation. This fact indicates that blue light evidently contributes not only to the intensification of synthesis of nitrogenous substances, but also to the mobilization and translocation of nitrogen from the nutrient medium to the young leaves.

TABLE 3

Nitrogen Content in Bean Leaves in Red and Blue Light

Light in experiment	Conditions of nitrogen nutrition	N in mg/100 mg of abs. dry wt.			Ratio of protein N to total N in %	N increase during supplementary feeding		Ratio of N in blue light to red light, in %	
		Total	Protein	Non-protein N		Total	Protein	Total	Protein
Red	1/4 nitrogen level	1.81	1.25	0.56	69	100	100	100	100
	Suppl. feeding with N up to full level	3.14	2.49	0.65	79	173	200	100	100
Blue	1/4 nitrogen level	2.80	2.35	0.45	84	100	100	154	188
	Suppl. feeding with N up to full level	3.92	2.89	1.03	74	140	123	124	116

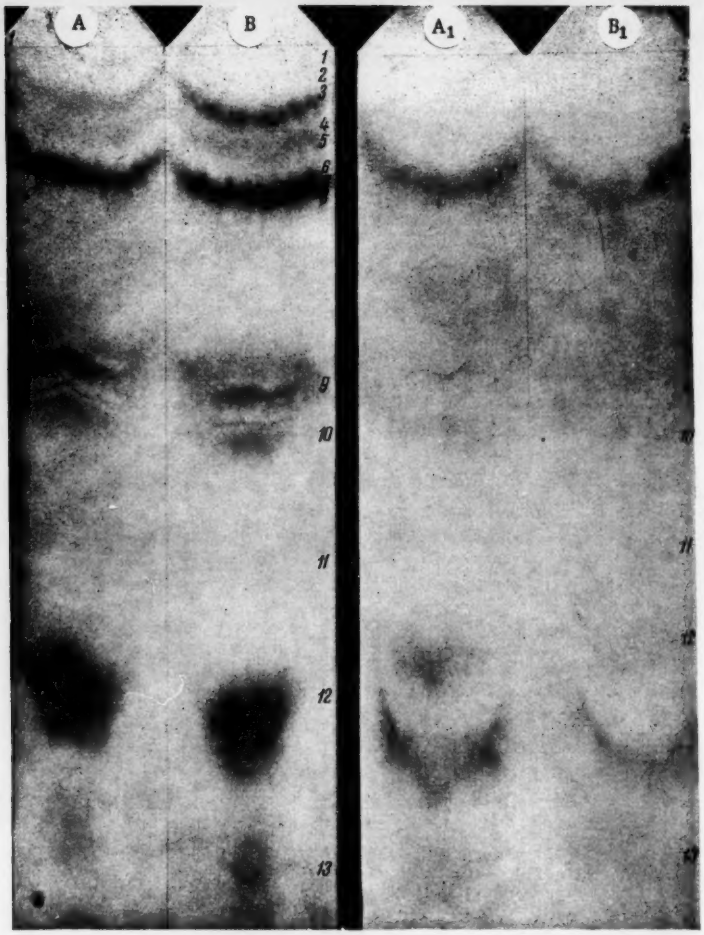

Fig. 1. Chromatogram of free amino acids during supplementary feeding with nitrogen (A and B) and during 1/4 nitrogen level (A_1 and B_1). A and A_1) red light; B and B_1) blue light.
Amino acids: 1) lysine; 2) histidine; 3) asparagine; 4-5) arginine and aspartic acid; 6-7) glycine and serine; 8) glutamic acid; 9) threonine; 10) alanine; 11) tyrosine; 12) tryptophan; 13) valine.

The action of blue light was particularly favorable to the accumulation of protein nitrogen. Both the amount of protein nitrogen as well as its ratio to total nitrogen was higher in the blue light during nitrogen starvation than in red light (blue light – 84%, red light – 69%).

On the other hand, the amount of nonprotein nitrogen under these conditions was lower for blue light. This, in particular, can be seen from the data of Table 3 and from the chromatogram of free amino acids (Figure 1). Amino acid content during nitrogen starvation was very small and the main mass of acids is represented by similar acids in both red and blue light – asparagine, glycine, and serine. At the same time, the color intensity of spots is higher for red light.

The supplementary feeding caused an increase in total and protein nitrogen content for both sources of illumination. The differences in organic nitrogen content favoring blue light remained. However, they were already significantly smaller than for starving plants, since the process of accumulation of organic substances at this time occurred more intensely in red light. This phenomenon was probably conditioned by a greated degree of initial starvation of plants in red light.

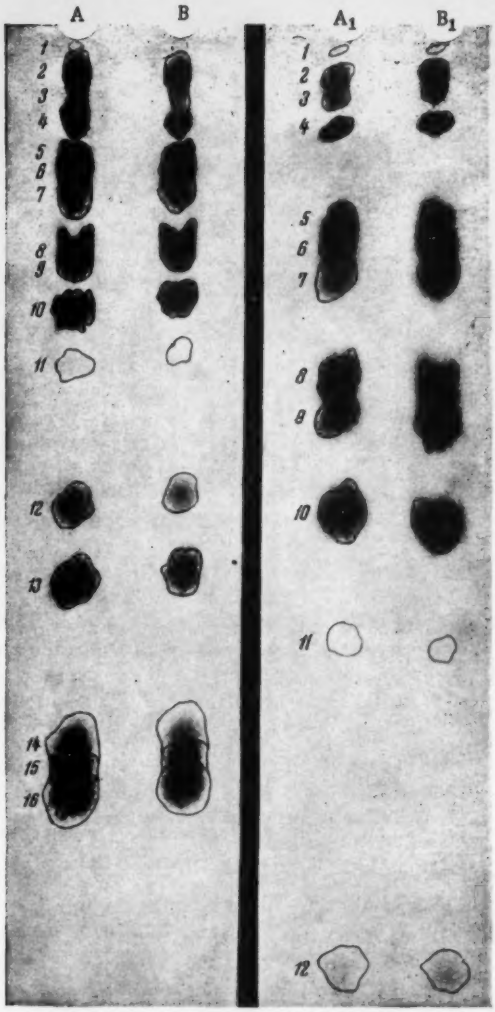

Fig. 2. Amino acid composition of the chloroplast protein hydrolyzate A and A_1 blue light: B and B_1 red light: A and B exposure duration 24 hours; A_1 and B_1 exposure duration 72 hours.
Amino acids: 1) cystine; 2) lysine; 3) histidine; 4) asparagine; 5) aspartic acid; 6) glycine; 7) serine; 8) glutamic acid; 9) threonine; 10) alanine; 11) proline; 12) tyrosine; 13) valine; 14) phenylalanine; 15) leucine; 16) norleucine.

The amount of nonprotein nitrogen, amino acids included, was significantly increased during supplementary feeding, to a large degree for blue light (see Table 3). In Figure 1, distinct spots of thirteen amino acids are seen. Their number is the same for both light sources. The intensity of staining of amino acid spots (with the exception of tryptophan) was higher in the blue light.

Besides nitrogenous substances, we determined the chlorophyll content in leaves (Table 4).

As can be seen from the data of Table 4, blue light contributes to chlorophyll accumulation in leaves during nitrogen starvation. Supplementary feeding with nitrogen led to the equalizing of chlorophyll concentration

TABLE 4

Light in the experiment	Conditions of nitrogenous nutrition	Chlorophyll content			
		in mg per 100 cm^2	in mg per g wet wt.	in % per 100 cm^2	in % per g wet wt.
Red	1/4 nitrogen level	1.76	1.10	100	100
	Suppl. feeding up to the full level	3.23	2.33	183	211
Blue	1/4 nitrogen level	2.84	1.46	100	100
	Suppl. feeding up to the full level	3.28	2.26	115	154

in blue and red light, through a less intense process of chlorophyll accumulation in blue light: during supplementary feeding in red light, the chlorophyll content was increased two times in red light, and 1.5 times in blue light. No differences were found in amino acid composition of the chloroplast protein hydrolyzate. The number of amino acids, as well as intensity of spot staining, insofar as could be judged visually, were the same in red and blue light (Figure 2).

The content of free carbohydrate (monosaccharides, disaccharides, starch) in leaves of experimental plants was changed not only in connection with the action of the spectral composition of light, but also according to the conditions of the plant's nitrogen supply (Table 5). During nitrogen defficiency, carbohydrate was high in both red and blue light. Small differences were found, favoring red light.

TABLE 5

Carbohydrate Content in Bean Leaves
(in mg sugar per 100 mg dry weight)

Light in the experiment	Condition of nitrogen nutrition	Monosaccharides	Sucrose	Starch	Total sum of sugars
Red	1/4 nitrogen level	3.67	2.92	18.71	25.30
	Supplementary feeding with nitrogen up to the full level	2.08	1.41	6.43	9.92
	Soil culture	Not done	Not done	4.95	–
Blue	1/4 nitrogen level	3.10	2.35	16.85	22.30
	Supplementary feeding with nitrogen up to the full level	1.10	2.71	8.11	11.92
	Soil culture	Not done	Not done	Traces	–

During supplementary feeding with nitrogen, the amount of carbohydrate sharply decreased in both red and blue light, more so in red light. As a result, the amount of these substances was already somewhat higher for blue light. Finally, under soil culture conditions in red light, up to 5% of starch was found, while only traces were found in blue light.

DISCUSSION

The most important result of our investigation seems to us to be the data concerning the changes in degree of action of the spectral composition of light on plant metabolism, in relation to the conditions of nitrogen nutrition.

The positive effect of blue light on the synthesis of nitrogenous substances showed up both in nitrogen starvation as well as in plants given supplementary nitrogen feeding (Table 3). However, a favorable effect of

short-wave radiation on protein synthesis had been shown particularly clearly during nitrogen deficiency in the nutrient medium.

In connection with this, one gets the impression that the effect of short-wave radiation is in some degree analogous to the action of high doses of nitrogen and that the positive effect of one factor may be, of course only within certain limits, substituted for by another. It is natural that the increase in the nitrogen level increases the synthetic possibilities for nitrogenous substances. Blue light intensifies the oxidative processes [21]. This circumstance may also have a positive effect on the synthesis of nitrogenous substances [22]. Thus, various causes – an increase in the amount of the original substances for the synthesis and a change in the conditions of the intracellular regime, show up externally in a similar way – in the increased synthesis of nitrogen-containing substances in the green leaf.

However, it seems to us that the favorable action of short-wave radiation on the synthesis of nitrogenous substances, particularly protein, is specific and will show up to a greater or smaller degree in any conditions when plants are supplied with nitrogen. This hypothesis is confirmed by the fact that earlier we [10], and also Kleshnin, Osipova, and Timofeeva [9], observed increased protein content in plants which were grown continually in soil culture with a good supply of nitrogen.

The chloroplast size in our experiment (Table 2) was greater in blue light. Similar data were obtained by Kandelaki [23]. It seems as though we should be able to speak of a specific effect of light quality for the size of chloroplasts, too.

However, from the data of Table 2 it can be seen that with improvement in the conditions of nitrogen nutrition (soil culture) the size of chloroplasts is increased in both red and blue light, and simultaneously the difference in their size is decreased for both sources of illumination. Besides, in our other experiments, growing beans continuously on two levels of nitrogen, we obtained a chloroplast length of the order 2.5-3 μ, for both blue and red light. Thus, at the high nitrogen level, simultaneously with the general increase in chloroplast size, the differences for red and blue light disappear. Because of that, it is very probable that the determining issue in the changes in chloroplast size in our experiments was not the illumination, but nitrogen nutrition, as has been indicated by Ulrich [24].

Coming to the analysis of data concerning chlorophyll accumulation, it should be pointed out that the action of the spectral composition of light on this process has been investigated mainly in connection with the rate of greening-up of the etiolated sprouts in various rays of the spectrum. The question of chlorophyll content in adult plants during an extensive growing period was studied much less extensively. However, the already existing material indicates the complexity of this question. There are indications of a more favorable action of long wavelength radiation on chlorophyll accumulation [11]; on the other hand, Stolwijk [20] did not find any effect of the spectral composition of light on this process. In plants which had been continually in the light of a mercury lamp (IGAR-2) and of neon tubes (ND-1), when the light flux was equalized according to the amount of incident quanta, no differences were observed in chlorophyll accumulation in red and blue light.

Chlorophyll content (in mg per g of fresh weight) in plants grown in red and blue light, turned out to be the following:

Plant	ND-1 ("red light")	IGAR-2 ("blue light")
Broad beans	2.10	1.93
	2.14	2.27
Sunflower	1.89	1.93
	1.83	1.71

Ermolaeva [11] and Kleshnin, Osipova, and Timofeeva [9] have obtained data which indicate that not all plants are the same in their response to short-wave and long-wave radiation. In the experiments of Ermolaeva [11], three of the plants investigated had a lower chlorophyll content in blue light (two varieties of perilla and tobacco) and one had a lower chlorophyll content in red light (fragrant tobacco). In the experiments of Kleshnin, Osipova, and Timofeeva [9] with two varieties of lettuce, the chlorophyll content was the same for the variety Berlinskii, independent of the intensity of the light in both red and blue light; while for the variety Maiskii, chlorophyll was always lower in blue light.

At the Second All-Union Conference on Photosynthesis, Pageeva and Brandt reported that, depending on the intensity of illumination, the accumulation of chlorophyll was increased either by red light (at lower illumination intensity) or blue light (at higher intensity).

In our experiments, we also had cases of dissimilar action of the light quality on chlorophyll accumulation. Sometimes blue light favored chlorophyll accumulation, but occasionally it had no effect on this process (Table 4).

Thus, there seems to be no single answer to the effect of the spectral composition of light on chlorophyll accumulation, evidently because the chlorophyll concentration results, as is known [25], from two simultaneously occurring processes – the new formation and the destruction of chlorophyll. The interrelation of these two processes may be determined for different plant groups by their species characteristics and their physiological state [26]. In this sense, short-wave radiation may be more favorable for the life of some plants, while long-wave radiation is more favorable for others. Because of this, in investigating this question, together with a statement of the fact of similar or dissimilar effects, one must attempt to clarify in each case the reasons which determine the direction of the process.

It seems to us that the data we obtained on chlorophyll accumulation speak of the close dependence of this process on the nitrogen nutrition of plants. During nitrogen deficiency, the determining factor for chlorophyll accumulation in leaves is possibly the protein concentration, since it is known that the basic amount of chlorophyll in the normally functioning leaf is connected with protein, and chlorophyll breakdown may be induced by a deficiency of the protein component of chlorophyll – the protein complex [27]. Because of this, the conditions which favor greater protein accumulation (blue light) also favored greater accumulation of chlorophyll. At the high nitrogen level (supplementary feeding), no parallelism is noticed in the increase of protein content and chlorophyll content of leaves, since not all protein, even in the chloroplasts, is found with chlorophyll [26]. Evidently, in this treatment a "saturation" of the reaction, in the sense of chlorophyll formation, was observed for both red and blue light, and it occurred faster than for the protein accumulation, since the content of the latter continued to increase.

Thus, in our conditions the leading factor in chlorophyll accumulation in leaves was found to be conditions of nitrogen nutrition. It is through this factor that the effect of light quality was also produced. When nitrogen was in the minimum concentration, the favorable action of short-wave radiation was obtained; during the absence of nitrogen starvation, the quality of light did not effect the accumulation of chlorophyll.

Amino acid composition of the chloroplast protein was similar both in red and in blue light, as analysis of the hydrolyzate showed (see Figure 2). However, these data do not remove the question of a specific effect of light quality on the properties of the protein-chlorophyll complex, since there are indications of greater stability of chlorophyll in blue light [28]. Since the chemical composition and structure of chlorophyll in cells are not the same in red and blue light, the reasons for differences in chlorophyll stability should evidently be sought in the change of the protein component of the complex. Because of this, it is possible that blue light increases the stability of chlorophyll toward destruction, changing the ratio between the processes of new formation and breakdown of chlorophyll in favor of new formation, not only through formation of a larger amount of the protein component, but also through a change of its physicochemical properties from those produced in red light.

The content of free carbohydrates in bean leaves is determined to a significant degree by the properties of the leaf in relation to synthesis of nitrogenous compounds. The smaller amount of carbohydrate is correlated with a greater content of nitrogenous substances in leaves, which indicates the use of carbohydrate for the building of the carbon chain of these substances.

From the point of view of the results obtained, it is easy to explain the data of those investigators who did not find a typical plant reaction to light in relation to carbohydrate accumulation: it is possible to observe cases of both positive and negative action of blue light on this process. The picture we observed of thecontrasting effect of light quality on the accumulation of carbohydrates was the result of differences in the nitrogen nutrition of plants. During nitrogen starvation, when synthesis of nitrogenous substances was slowed down and the growth processes were inhibited, the carbohydrate accumulated in significant amounts in both red and blue lights. With breakdown of the equilibrium in nitrogen nutrition (supplementary feeding with nitrogen), the amount of carbohydrate in red light was smaller than in blue light, evidently because of the more intense synthesis of nitrogenous

substances for this period of time in the red light, and also, possibly, through more rapid growth of plants. Finally, at the prolonged high level of nitrogen in the nutrient medium and the intense process of growth and accumulation of organic substances, when a "threshold of saturation" was evidently reached in the plant for the given condition (soil culture), there was little carbohydrate in the leaf. A higher level of "saturation threshold" for protein synthesis in blue light evidently can explain the smaller carbohydrate content under these conditions [10].

Summing the materials obtained in relation to action of the spectral composition of light on several indicators of metabolism, one may say that blue light specifically affected the accumulation of nitrogenous substances in bean leaves, increasing this process. Its positive action was particularly sharply shown during nitrogen deficiency. From this, it may be concluded that short-wave radiation favors not only synthesis and accumulation, but also the mobilization of nitrogen from the nutrient medium.

The greater chlorophyll accumulation and the greater size of chloroplasts in plants in blue light during nitrogen deficiency in the nutrient medium may evidently be connected with the more favorable conditions for the mobilization of mineral nitrogen.

Some time ago, Liubimenko [29] proposed that, together with the primary photochemical reaction of photosynthesis, secondary photochemical reactions are taking place in leaves. The change of spectral composition of light changes the ratio between these reactions, thus influencing the process of transformation and assimilation of metabolites. Lately, the significance of secondary photochemical reactions for assimilation of carbon and carbon dioxide during photosynthesis was also indicated by Nichiporovich [30].

It is possible that the significance of at least some secondary photochemical reactions, according to the terminology of Liubimenko, induced by short-wave radiation, consists in their direct or, more likely, indirect effect on the rate of synthesis of nitrogenous components.

SUMMARY

1) Growing bean plants in red and blue light (under conditions of similar rates of accumulation of dry organic mass by the plant) brought about changes in several biochemical and anatomical indicators.

2) Blue light, as compared with red, favored the synthesis of nitrogen-containing organic substances, and increase of chloroplast size, and an increase in chlorophyll content.

3) The degree of the differences depended upon conditions of nitrogen supply to the plants. During nitrogen deficiency in nutrient medium, all differences in favor of blue light had been considerably more sharply shown. With the improvement of nitrogen nutrition, these differences were leveled off.

4) The carbohydrate content is correlated with the rate of accumulation of nitrogenous substances in leaves. The rapid accumulation of the latter was accompanied with a decrease in carbohydrate content, independent of whether this process was observed in red light or in blue light.

5) It is proposed that the basic reason for all indicators studied was the positive effect of short-wave radiation on the synthesis of nitrogenous substances.

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*In Russian.

**See English translation.

***Russian translation.

CONCERNING THE PHOTOSYNTHESIS OF POTATO UNDER CONDITIONS OF THE FAR NORTH

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The 14 hours of daylight during the month and a half of summer, and the low temperatures throughout the growing season, make the regions of the far north a "natural laboratory" [1] for the solution of many important questions in plant physiology. One of the most interesting questions is that of the possibility of utilization of nighttime illumination by arctic plants.

Earlier [2, 3] as well as later investigations [4-7], have established that plants which grow under the conditions of the long polar day had a greater dry weight than plants which received an artificially shortened photoperiod. However, these experiments left unclear during which period of night – in the late evening or, on the contrary, in the early morning hours – the light is used by the plants on the long day for the supplementary synthesis of dry matter, and whether assimilation is continued in them even at midnight, during minimal illumination intensity.

Attempts to determine the existence of night photosynthesis by the stomata [8] were also not successful, since it was established [9] that the stomata in various species of arctic plants may open and close at altogether different times of the day. Only the papers of Kostychev et al. [10], Müller [11], and lately of Dadykin and Grigor'eva [12] proved that 24-hour-a-day photosynthesis during the polar day was possible in a number of arctic plants – polar birch, willow, cloudberry. Insofar as cultivated plants are concerned, no assimilation was observed during the night hours in two species studied in this connection – potato and corn [12, 13].

The data on the rate of productivity of plant photosynthesis in northern latitudes are contradictory. According to Kostychev [10], the rate of assimilation in the north is somewhat lower than in the temperate zone. According to the data of other authors [11, 14-16], the rate of assimilation in arctic and temperate latitudes is approximately the same. Finally, according to the data of Goncharik [17], the rate of photosynthesis in the far north is considerably higher than in the temperate zone, which the author considers to be an adaptive reaction of plants to the shortened growing season in the north.

Thus, in spite of the fact that the investigations in the field of plant photosynthesis in northern latitudes began a long time ago, until now the following have not been clarified:

a) Are all plants capable of assimilation during night hours, or only the representatives of the polar flora?

b) What is the role of night photosynthesis in the total production of organic matter in the north?

c) Do plants in northern latitudes, because of their night assimilation, have any advantage over plants of temperate latitudes?

Finally, the question of the values of rate and productivity of photosynthesis in the north needs further study.

In the present work, an attempt was made to study the peculiarities of photosynthesis in the north and to understand the changes which take place in the course of this process in northern latitudes as compared with temperate latitudes. In connection with this problem, we carried out work in two places – in Moscow and in the north, in the region of the Kol'skii Penninsula (Station Apatity – Lat. 67° 41' N). Two strains of the potato variety Early Rose were taken as the experimental objects, one grown in the north for more than twenty years and another which was brought from Moscow annually. Such an experimental setup made it possible to carry out an investigation not only with respect to geographic areas but also to clarify those possible physiological changes which may take place in plants of temperate latitudes when they are transferred to northern latitudes.

The work was carried out in 1955 and 1956.

METHODS

Potatoes were usually planted as vernalized tubers by the square hill method (70 × 70 cm) in the beginning of June (June 7-14) in the north and in the middle of May in Moscow. Before planting, fertilizers were applied to the soil – organic (manure or peat), at the rate of 15-30 tons/hectare, and mineral (NPK), at the rate of 60 kg/hectare of the active principle of each element.

The photosynthetic rate was measured by the method of Ivanov and Kossovich [18] every five yours throughout the 24-hour period, beginning at 7 A.M. (Moscow time). The duration of exposure was from 5 to 10 minutes in the day time and up to 15 to 20 minutes in the evening and at night. During exposure, the flasks with leaves were placed in the well-illuminated section of the field, not shaded by other plants. All determinations were made on leaves on the middle part of the plant and, in 1956, also on the upper leaves.

At the same time, the dynamics of dry-matter accumulation by tubers and aerial plant organs was determined. Samples of 20 plants were taken every 10 days throughout the whole growing season.

Duration and Diurnal Rhythm of Photosynthesis in the North

The results of two years of determinations (part of which are given in Figure 1), which included various weather conditions and phases of development, allowed us to note the following characteristics of photosynthesis in the north.

1. The diurnal rhythm of photosynthesis in almost all experiments was expressed by a one-peaked curve with a maximum at noon and a minimum at about 10 P.M. It is interesting to note that in approximately one third of the experiments photosynthesis already reaches close to maximal values in the early morning hours (7 A.M.), and towards noon only an insignificant increase takes place (Figure 1, A and B). According to the concept developed by a number of investigators [19-20], the basic reason for the noon depression of photosynthesis is a water deficit, created in assimilating tissues under the influence of prolonged intense illumination, high temperature, and deficiency of moisture in the soil.

In the region of the Kol'skii Penninsula, where the number of cloudy days and of days with changeable cloudiness is very great, and the air temperature as a rule is lower than 30°, the noon depressions are observed relatively seldom, in spite of the prolonged (over the course of a month and a half) 24-hour-a-day illumination.

2. Towards 10 P.M., the rate of photosynthesis is sharply decreased, but towards 3 A.M. increases again up to 2-4 mg CO_2/dm^2/hour. To determine how assimilation takes place in the period between 10 P.M. and 3 A.M., and whether it continues even at midnight, in 1956 we carried out a series of experiments in which photosynthetic rate was measured every 1.5 hours, beginning at 10 P.M. These experiments continued throughout all of July, i.e., the period with a day length from 24 hours down to 19 hours 20 minutes. The results of these experiments are given in Figure 2. It is easy to note that during the period of the 24-hour polar day in the Apatity region, until July 10, potato photosynthesis is continued throughout 24 hours, at midnight reaching 1.0-1.8 mg CO_2/dm^2 per hour and towards 4 A.M. increasing up to 4-11 mg (Figure 2, A and B). On July 19, after a decrease of day length to 21 hours, and a certain weakening of illumination intensity during the night hours, photosynthesis still continued throughout 24 hours but its rate at midnight was reduced to 0.7-0.9 mg CO_2/dm^2 per hour, and its increase at 4 A.M. was already not so considerable – 3 to 4 mg CO_2 (Figure 2,B). Finally, during the observation at midnight on August 1, photosynthesis was absent.

Thus, the period of continuous assimilation of the potato in the north is terminated between July 20-30. (It is necessary to note, however, that all night experiments, except the experiment on July 3-4, had been carried out during clear weather. During cloudy weather, the period of continuous assimilation undoubtedly may be terminated still later.)

If we turn to the experiment carried out in the period of the longest day in Moscow (17 hours 34 minutes), we see that photosynthesis ceases there between 9 and 10 P.M., i.e., almost immediately after sunset (9:19 P.M.), and begins only with the sunrise (3:34 A.M.), towards 4 A.M. reaching only 1.6-2.7 mg CO_2/dm^2/hour (Figure 2,E).

From the data of the experiment we see that the duration of assimilation in potato in the north during the polar day is approximately 7 hours longer than in the temperate latitude in the period of the longest day.

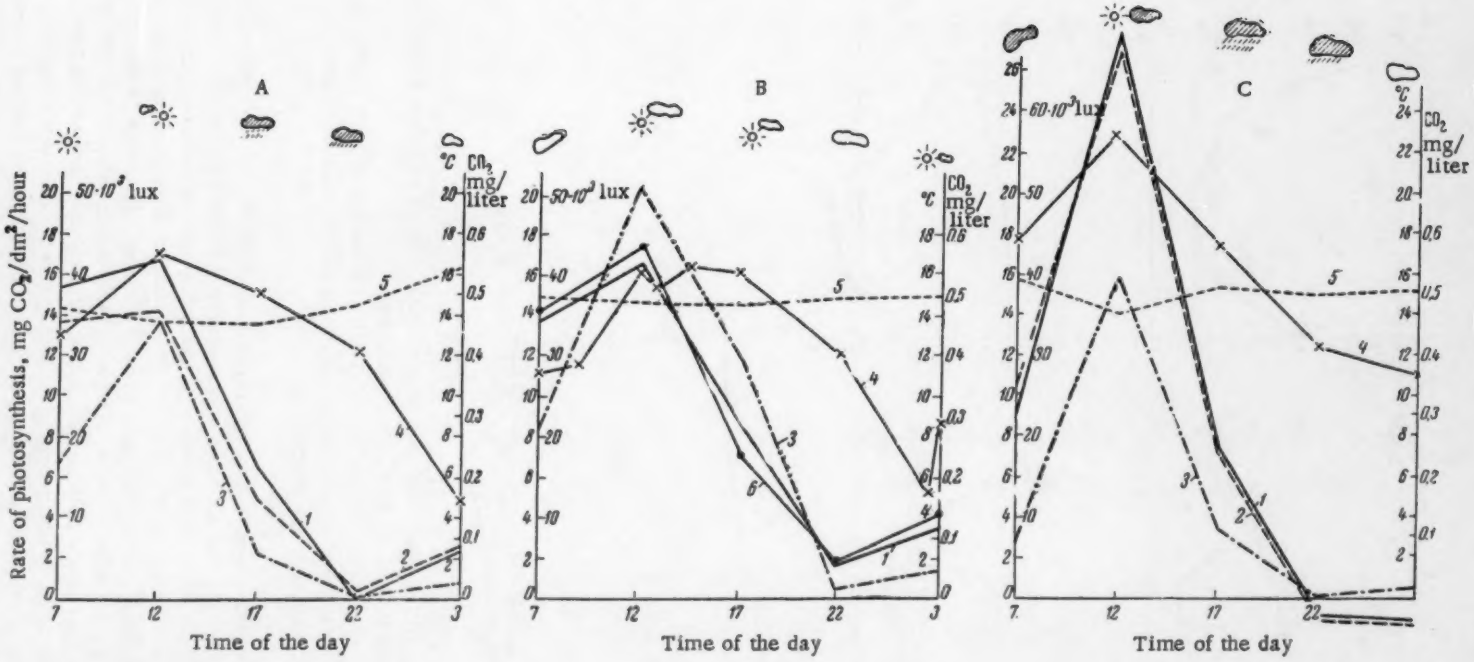

Fig. 1. Diurnal course of potato photosynthesis in the far north (Station Apatity) and in Moscow.
A) Phase of flower bud formation. Apatity, July 1-2, 1955; B) phase of the beginning of flowering, Apatity, July 25-26, 1956; C) phase of flower bud formation, Moscow, June 28-29, 1956; 1) potato variety Early Rose, northern strain; 2) Ranniaia rosa, Moscow strain; 3) intensity of illumination; 4) air temperature; 5) CO_2 content of the air; 6) Early Rose, northern strain (upper leaf).

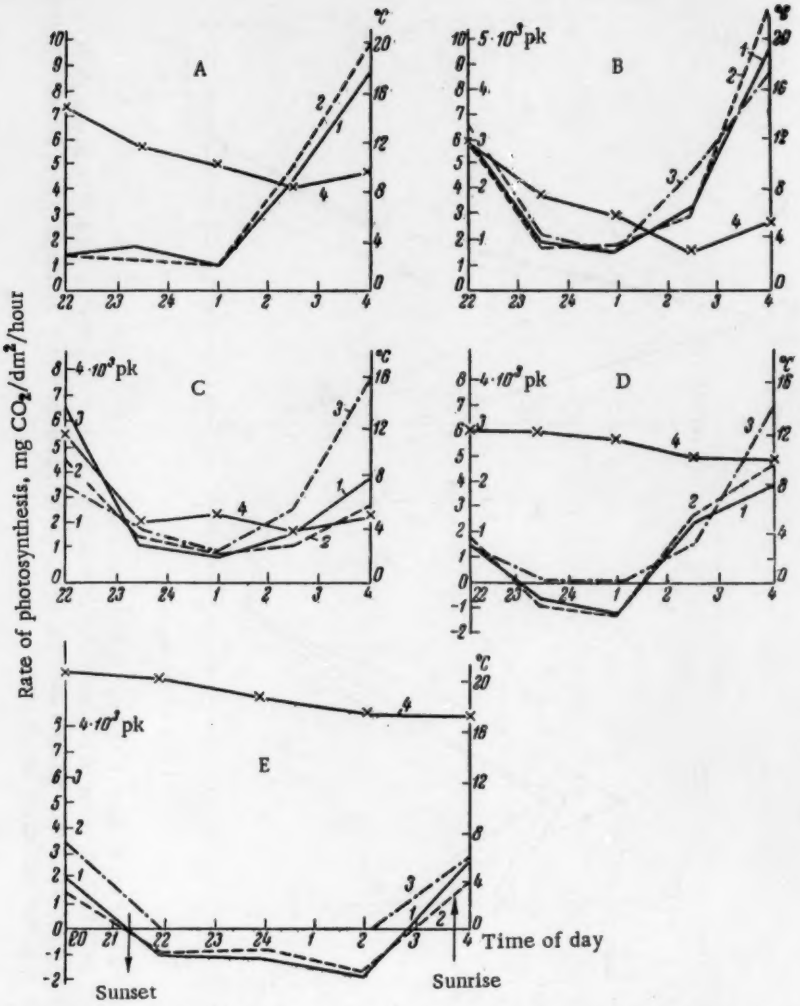

Fig. 2. Dynamics of absorption and evolution of carbon dioxide in potato in the far north (Apatity) and in Moscow during the night hours.
Apatity: A) July 3-4; B) July 6-7; C) July 18-19, D) July 31-August 1; E) Moscow June 23-24; 1) potato variety Early Rose, northern strain; 2) Early Rose, Moscow strain; 3) intensity of illumination; 4) temperature.

The data obtained allow one to conclude that not only wild arctic plants, but even potatoes, may assimilate continuously during the polar day. The absence of night photosynthesis in potatoes and corn in the experiments of Dadykin [12] and Gorbunova [13] may, in our opinion, be due to a number of causes: 1) insufficient intensity of illumination during night hours at latitude 62°, where the investigations of these authors were carried out; 2) the late time of the determinations. Part of the experiments of Gorbunova [13] were carried out with potato in August, when the period of continuous assimilation is terminated in the potato between July 20-30, as was shown by us earlier, even at the latitude of 67.5°. Evidently at latitude 62°, it should terminate earlier; 3) insufficient sensitivity of the method of photosynthesis determination in the experiment of Gorbunova—Sachs' method of leaf halves. Finally, a possible cause of the absence of night photosynthesis may be the lowered temperatures of the air and soil during night hours in the region of Igarka, as compared to the region of Kol'skii.

The comparison of northern and Moscow strains shows that plants just brought from the temperate belt are, under conditions of continuous illumination, capable of utilizing the night light to the same degree as plants grown in the north for a long time (Figure 2).

Thus, during continuous illumination over the course of a whole month, potato plants in the northern latitudes retain the definite rhythm of this process in the course of individual days, which to a large degree is determined by the daily course of illumination intensity. The maximum assimilation is observed at noon, the minimum – at midnight.

Rate and Productivity of Photosynthesis in the North

In various treatments in the experiments of 1955, the maximum values of photosynthesis in the north during the noon hours reached from 18-24 mg CO_2/dm^2 per hour in the sprouting phase and from 14-17 mg in the phase of flower-bud formation (Figure 1,A). During the morning and afternoon hours the rate of photosynthesis varied from 5-10 mg CO_2/dm^2 per hour.

In 1956, the values of photosynthetic rate were somewhat lower, possibly because of the drought which occurred in July. In the leaves of the middle zone of the plant, the maximum values reached 14-16 mg CO_2/dm^2 per hour and in the upper leaves, 17 mg (Figure 1, B). No significant difference in assimilation between upper and middle leaves was observed, evidently because the distance between them was small – only 3 to 4 leaves.

TABLE 1

Average Daily Yield of Photosynthesis in Potato in the North (Apatity)
(in mg CO_2/dm^2 per day)

Year	Experimental treatment	July	Aug.	Sept.
1955	Early Rose, northern strain	215.6	131.8	22.6
	Early Rose, Moscow strain	166.5	116.0	22.0
1956	Early Rose, northern strain	206.3	121.0	—
	Early Rose, Moscow strain	157.3	141.6	—

TABLE 2

The Amount of Carbon Dioxide Assimilated by Potato for 6 Night Hours Under Northern Conditions (Apatity) in 1956
(in mg CO_2/dm^2 per 6 hours)

Experimental treatment	July 3-4	July 6-7	July 18-19	July 31-August 31
Early Rose, Northern strain	18.3	22.0	13.0	5.5
Early Rose, Moscow strain	19.8	22.5	10.6	5.9

In Moscow, maximal values of assimilation reached 27-28 mg CO_2/dm^2 per hour during the phase of flower-bud formation (Fig. 1,B). According to the data of L. E. Stroganova over many years, the maximal rate of photosynthesis in the potato fluctuated under the conditions of the Moscow region, averaging 25 mg CO_2/dm^2/ hour during the first half of the growing season, only in September decreasing to 14-20 mg. From this, we must conclude that the rate of photosynthesis in the potato in the north is somewhat lower than in the temperate belt. However, if we turn to the figures of the average daily productivity of photosynthesis according to carbon dioxide utilization, we see that they are almost equal in Moscow and in the north (Table 1).

From Table 1, it can be seen that the average daily yield of photosynthesis in the north varies in the northern strain of Early Rose, around 200 mg CO_2/dm^2/day in July, and about 130 mg per day in August. In September, the daily yield of photosynthesis is sharply decreased.

In the Moscow region, the average daily yield of photosynthesis, according to unpublished data of L. E. Stroganova, also varies around 200 mg in June and July and about 150 mg in August. The yield of photosynthesis in the Moscow region is rather high even in September – 108 mg CO_2/dm^2 per day.

Thus, in spite of a somewhat decreased rate of photosynthesis as compared with the northern latitudes, the potato in the north has approximately the same average daily yield.

The high daily yield of photosynthesis in the north is assured, first, by the presence of continuous photosynthesis during the polar day. It can be seen from Table 2 that, for six hours of light during the night, the potato plant assimilates from 18-22 mg CO_2 (on the average 20.7 mg), which makes about 10% of the total daily yield of photosynthesis, with a subsequent decrease during shortening of the day length down to 5.7-2.7%. The high daily yield of photosynthesis in the north is assured, secondly, by the one-peak type of curves of the diurnal rhythm during rapid photosynthesis in the early morning hours. From Figure 2, we have already seen that at 4 A.M., when photosynthesis in Moscow is only beginning and reaches only 1.6-2.7 mg CO_2/dm^2 per hour, in the north it rises to 4-11 mg CO_2.

Values of the yield, calculated according to the amount of carbon dioxide absorbed per day, with a coefficient of efficiency of 0.3, coincide relatively well with figures of the net yield of leaf surface, calculated from samples of dry matter for definite time periods. This can be seen in Figure 3, where the curve presents the course of changes of the net yield of photosynthesis and the circled points represent the yield calculated by carbon dioxide uptake. From the same Figure 3, it can be seen that the value of net photosynthetic yield in the north varied from 6 to 3 g of dry matter per m^2 per day, i.e., within the same limits as in the temperate belt. Thus, the analysis of data on the dynamics of accumulation of dry matter, similarly to the analysis of data on photosynthesis, brings us to the same conclusion – that the average daily yield of organic substance by potato plants is approximately the same in the north and in the temperate belts.

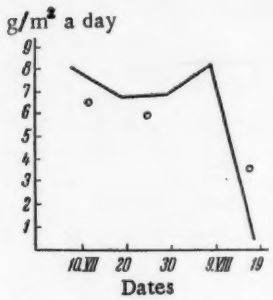

Fig. 3. Change of the net photosynthetic yield in potato in the course of growth in the north (Apatity).
Experiment in 1956.

However, it must be noted that the period of active photosynthesis with high daily yield in the north is much shorter than in the temperate belts, and varies in different years from 40 to 50 days, on the average about a month and a half (July and one-half of August), while in the temperate belt it is from 3 to 3.5 months (June, July, August and one-half of September). The shortening of the period of active photosynthesis is compensated in potato up to a certain degree by the good development of leaf surface, which is evidently favored by continuous illumination [21]. As a result of good development of leaf surface and high daily yield of photosynthesis, the final yield of tubers in the north may reach 180 and even 200 centners/hectare, in spite of the great shortening of vegetative period.

One may judge the importance of photosynthesis in yield formation in the north by comparing the results of our experiments of 1955 and 1956. In 1956, the leaf surface was relatively small and reached only 22,000 m^2/hectare; the period of active photosynthesis was about 60 days. In 1955 the leaf surface reached 40,000 m^2/hectare, but, because of the late appearance of sprouts, the period of active photosynthesis was greatly shortened. Because of this, the final yield of tubers in 1955, in spite of good development of leaf surface, was still lower than in 1956, – 157 centners/hectare instead of 185 centners/hectare.

One must be careful in drawing conclusions from a comparison of the northern and Moscow strains. According to the experiments of 1955, plants which were just brought to the north had a somewhat lower daily photosynthetic yield as compared to the local form (Table 1), which evidently could have been a direct reaction of the organism to unfavorable conditions of existence. In 1956, the experiments with the local and introduced forms were carried out on different days; because of this, the observed differences in the value of daily yield may have been due to dissimilar weather conditions at the time of the experiment.

The comparison of the diurnal course of photosynthesis with the course of illumination intensity in the north exposed, in almost all experiments, the relationship between the daily dynamics of phtosynthesis and illumination. In the leaves of the middle zone of the plant, within the limits of low illumination (up to 10-12 thousand lux) there was an almost linear relationship between illumination intensity and photosynthesis rate (Figure 4). Light saturation of the middle whorl, developed under certain shading conditions, took place in the north at 16-18 thousand lux, which is much lower than in the temperate belt. (According to the unpublished data of L. E. Stroganova, under Moscow conditions this occurs only at 40 thousand lux.)

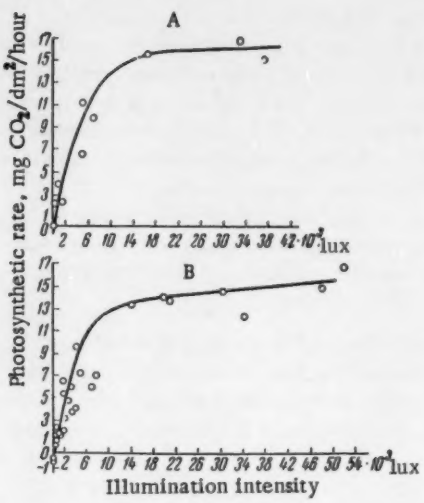

Fig. 4. The light curve of photosynthesis. A) According to the data of 1955, and B) according to the data of 1956.

Comparison of the photosynthetic rate with the course of illumination shows, also, that the value of assimilation and of low-light assimilation is higher in the north than in the Moscow region. Thus, for example, in Moscow at a 20,000 lux illumination intensity, the assimilation rate reaches 1-2 mg/dm² per hour (Figure 2). In the north at approximately the same illumination, assimilation may reach 3-4 and even 6 mg CO_2/dm² per hour. Even at midnight, at an illumination intensity of only 500 lux, assimilation reached 0.7-0.9 mg CO_2/dm²/hour.

The compensation point in the north also lies relatively low, at 220-260 lux. Evidently such a low position for the compensation point and the threshold of light saturation in the north is a response reaction of the plant to lower illumination intensity in northern latitudes, as well as to certain shading conditions, in which the leaves of the middle whorl of the potato plants with which we experimented were developed. An analogous picture was observed in the experiments by Burnside and Böhning [22]: during growth of such light-requiring plants as sunflower, cotton, tobacco etc., under conditions of shading, the light saturation and compensation points occurred significantly lower than in those plants which were grown in full sunlight.

Comparison of the course of photosynthesis with the temperature curve allowed clarification of the significant assimilation at lower temperatures. Thus, in the experiment of July 6-7 (Figure 2, B) at a temperature of 3.3°, assimilation reached 2.9-3.2 mg CO_2/dm² per hour, and in the experiment of July 15-16 at a temperature of 1.0°, it reached 1.0 mg CO_2. Whether such a capacity for assimilation at lower temperatures is a characteristic peculiarity of plants of especially northern latitudes, it is as yet difficult to say. It is known [23] that plants of high altitudes, living under more severe conditions of existence, have a very low temperature limit for photosynthesis, and may assimilate even at −8 to −10°.

It is possible that in the northern latitudes the plants adapt themselves to the utilization of light of low intensity and at low temperatures.

DISCUSSION OF RESULTS

From the experiments given above, we saw that, in spite of the facts given in the literature, during the polar day in the far north the potato may assimilate continuously, and that the night assimilation makes up about 10% of the total daily yield of photosynthesis. Naturally, the question arises whether this value is real and what does it give to the plants? If the period of night photosynthesis is taken to be 6 hours (from 10 P.M. to 4 A.M.), and the duration of continuous assimilation is approximately 25 days (from the time of sprout appearance, from the beginning of July to July 25), then taking into account the change in leaf area and the decrease in photosynthetic rate during the night hours when the day length is shortened, we can calculate that one potato

plant assimilates over that time about 20.5 g CO_2, and that the plants on 1 hectare assimilate up to 415 kg CO_2. As can be seen, the value is rather considerable. However, in the temperate belts, potato plants daily assimilate 150-250 kg CO_2/hectare. Thus, the amount of carbon dioxide which plants in the north assimilate over the whole period of night photosynthesis is assimilated by the plants of temperate latitudes in two or three days.

Night assimilation, taken as such, evidently does not play an essential part in the total production of organic substances in the north. However, significant assimilation during the early morning hours, together with night assimilation, gives a very substantial production of organic matter. This evidently conditions the fact that the long day in the north compensates the decrease in photosynthetic rate arising through lower temperatures and lower illumination. However, from the above it is completely clear that the long day cannot completely compensate for the strong shortening of the growing season in the north, the result of which is that the final yield of tubers in the northern latitudes is always somewhat lower than in the temperate latitudes. The relatively low rate of assimilation during the night hours (maximal – 1.8 mg CO_2/dm^2 per hour; minimal – 0.7 mg) is conditioned by the low intensity of the nighttime illumination (Figure 2), which was not noted as yet by Razumov [24]. During the daytime hours, the limiting factor may be temperature, as well as intensity of illumination.

Finally, it is necessary to point out that the conclusions we made concerning the time of termination of continuous assimilation, concerning the shape of the curves of the diurnal course of photosynthesis and of the values of daily yield, we do not apply to other plants nor to other regions of the far north because of particular climatic conditions on the Kol'skii Penninsula. It is possible that in other regions of the far north adaptations to the shortened growing season take place in other ways – for example, by way of increase in photosynthetic rate during the same duration of the process as in temperate latitude, as it occurred in the work of Goncharik [17] and Gorbunova [13].

SUMMARY

The diurnal course of photosynthesis in potato plants cultivated in the far north (Kol'skii Peninsula) is usually described by a one-peaked curve with a maximum approximately at noon and a minimum at midnight.

During the 24-hour period of polar daylight (up to July 10) potatoes of northern origin as well as those transported from Moscow assimilate throughout the whole day, the rate at midnight being 1.0-1.8 CO_2/dm^2/hour. Assimilation at night was up to 10% of the total daily photosynthetic productivity.

Assimilation did not cease during the night when the day length decreased to 21 hours at about July 20; however the rate of photosynthesis at midnight dropped to 0.7-0.9 mg CO_2/dm^2/hour.

Only between July 20 and July 30 did assimilation over a complete 24-hour day cease in potatoes cultivated on the Kol'skii Peninsula.

Although the photosynthetic rate of potato plants in the north is somewhat smaller than that observed in Moscow, the mean daily yields of photosynthesis are approximately the same in both cases. In July it was 206-215 mg CO_2 per day; in August, 120-130 mg or 6-8 grams of dry weight per square meter per day.

In conclusion, I should like to express my deep gratitude to M. G. Zaitseva and L. E. Strogonova for valuable advice and direction made in the course of the present work.

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REMOVAL OF VERNALIZATION CHANGES IN THE PROCESS OF VEGETATIVE REPRODUCTION OF PLANTS

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At the present time, it is customary to consider that changes occur in the meristem during vernalization, as a result of which qualitatively different tissues and organs may arise [1]. Less clear is the question as to whether these changes may be removed, so that the plant would become again incapable of fruit-bearing without a repeated vernalization, as it happens during the formation of new seeds.

Avakian [2] showed that such removal of vernalization changes takes place during the formation of special "air bulblets" in the inflorescences of viviparous forms of onion . However, it remains unclear whether removal of such changes can take place during typical vegetative reproduction of the plant.

Experiments with grafting of metameric stem sections carried out by Lysenko with soybean and cotton [2], by Pogosian with wheat [3], by us with rice [4], and by several other authors with different plants, have shown that the higher the stem section, the faster a plant formed from it reaches a reproductive stage. These facts give us reason to suppose that the lower parts of the stem have the properties of the first stages of development, while the upper ones have those of the final stages of development. This series of experiments had also caused us to believe that only accumulation of the phase changes takes place during formation of the sprout, without any "removal" of those.

On the other hand, the experiments of a number of authors, those we summarized in 1943 [5], as well as those which were published later [6-9 et al], gave us reason to suppose that under certain conditions vernalization changes may be removed to some degree not only during seed reproduction but also during vegetative reproduction. However, all these experiments were carried out under conditions of development of one sprout without obtaining a new plant, and because of this, they did not give a direct answer to the question indicated above.

The study of the biology of vegetative reproduction of perennial cereals made available new facts which we shall report.

Experiment I. The experiment was carried out with plants of wild perennial rye, Secale anatolicum, isolated by us by the presence of so-called elongated ("sterile") stems, which have already been described in such cereal species as barley, timothy, and tall rye grass [10-13 et al.]

Since such stems have not yet been described in perennial rye, we shall give a short characterization of our plants. They were found in the summer of 1953 as 8 individuals (1.3% of the total population). In all eight plants there were 265 of the usual reproductive stems and 51 elongated vegetative stems. The fraction of elongated vegetative stems in individual plants varied from 3.4 to 42.9% (Figure 1).

A comparative analysis of reproductive and elongated vegetative stems showed the following most essential differences:

1. The number of internodes in the reproductive stems was within the limits of 6-7, while in the elongated vegetative stems it was no lower than 16, and in some cases it reached 25-30.

2. The height of reproductive stems in the period of seed maturation was 94 cm on the average; at the same time elongated vegetative stems were 112 cm in height, while towards the fall they grew to 165-180 cm.

In the beginning of autumn the upper, newly appearing internodes in the elongated vegetative stems turn out to be shorter, as a result of which the top of such a stem began to look like normal vegetative shoot developing from the rye seed. In some such stems the top began to branch, forming a unique aerial "branching node," morphologically similar to the usual node of tillering. The formation of such aerial nodes takes place from the apical meristem and is very similar to the formation of the usual tillering node which takes place after seed sprouting.

Fig. 1. General view of Anatolian rye, having simultaneously reproductive and elongated vegetative sprouts.

Fig. 2. The general appearance of the plant with the formed stem, bearing a head and two aerial vegetative shoots.

Fig. 3. The tip of the reproductive stem with head and new vegetative shoots.

TABLE 1

Characteristics of Elongated Vegetative Stems and Nodes of Branching Arising on Them

Number of stem	Characteristic of the original elongated vegetative stem		Number of shortened vegetative shoots in the "node of branching" forming on the tip of the given elongated vegetative stem
	height in cm	number of internodes	
1	180	21	2
2	170	16	3
3	165	17	1

Three elongated vegetative stems which were characterized on October 17, 1953 by the data given in Table 1 were taken for the experiment.

To clarify whether these shoots are "winter" or "summer" shoots, the nodes which formed them were cut, and the shoots forming there were planted in pots which were at once transferred to the room, thus being isolated

Fig. 4. Diagram of the structure of the reproductive stem with new vegetative shoots arising on it.

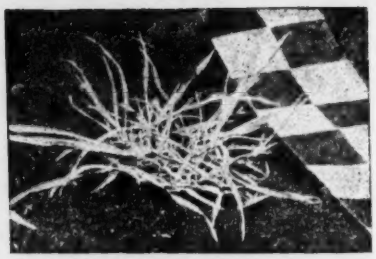

Fig. 5. General appearance of perennial rye plant developed from the shoot which was formed in the upper node of the stem shown in Figures 2, 3, and 4 (Photo June 1, 1955).

Fig. 6. General appearance of Anatolian rye plants on June 17, 1955.
1) Plants from normal sprouting shoots, forming near the base of the old stem;
2) plants from the aerial nodes, formed on the tips of elongated vegetative stems.

TABLE 2

The Presence of Lower Temperatures During the Autumn of 1953, to the Moment of Transfer to Cut Shoots into the Room

Temperature indicator	Number of days with air temperature	
	lower than 10°	lower than 5°
Daily average	44	26
Minimal	77	39

from the vernalizing action of lowered natural temperature. The plants were kept in a warm room over the course of the whole winter of 1953-1954. On April 20, 1954, after warm weather had become established, all three plants were transplanted from the pots into the soil.

For characterizing the possibility of natural vernalization of shoots during their growth period, we give the following data (Table 2).

As can be seen from Table 2, prior to transfer into the room, all shoots could have been vernalized to a significant degree. Nevertheless, throughout the whole vegetative period of 1954 all three plants remained in the

TABLE 3

Characteristics of Plant Grown From the Tip Nodes of Branching

No. of plants	No. of living shoots in tillering phase, 9/29/54	Height of plant in cm, 7/22/55	No. of stems on the plant, July 22, 1955		
			reproductive with normal head	elongated vegetative without a head	elongated vegetative, giving underdeveloped head
1	115	145	104	26	9
2	35	125	31	2	0
3	110	150	66	1	6

TABLE 4

The Presence of Lowered Temperatures in the Greenhouse During the Autumn of 1954

Temperature indicator	Number of days with air temperature	
	lower than 10°	lower than 5°
Daily average	23	13
Minimal	45	20

tiller phase and did not form a single elongated stem, while the original maternal plants, which wintered in the soil as during the first year of life, had reproductive as well as elongated vegetative stems. One must, by the way, dispose of the hypothesis that growing the plants at low light intensity during the winter may, by itself, cause such exhaustion that they altogether lose the capacity to head. In our experiments, growth of summer forms took place under the same conditions, and no retardation of their heading was noticed.

The number of shoots on each plant were counted on September 29, 1954. After wintering, the plants continued vegetative growth and formed normal stems which were counted and cut during ripening of the seeds (July 22, 1955). The results of all of these analyses are given in Table 3.

After cutting the stems, the usual production of short vegetative shoots began in the plants.

There is a good basis for supposing that elongated vegetative stems appear in rye only after their growing points terminate their vernalization. This is supported by the fact that our plants, which did not head in 1954, formed both reproductive and elongated vegetative stems in the summer of 1955, after wintering under natural conditions.

If one concedes that the elongated vegetative stem develops only after complete vernalization of the terminal growing point which gave rise to it, then one must say that the apical meristem and the tissues which were formed by it in such shoots are in a vernalized state. Consequently, even new shoots formed in the node in the autumn would turn out to be "summer" shoots, and because of this, without vernalization must form elongated vegetative, and perhaps even the usual reproductive, stems. However, as was mentioned above, all three plants, in spite of the relatively long vernalization during the fall, behaved as typical winter plants until their transfer into the room, and formed heads only after receiving repeated natural vernalization during the winter of 1954-1955.

Thus, the vernalized meristem gave rise in a purely vegetative way to new shoots, "winter" shoots in nature. The changes due to vernalization turn out to be completely, or at least to a very large degree, removed.

Experiment II. In the fall of 1954, in one of the vegetative experiments set up for the purpose of studying the dynamics of shoot formation in perennial cultivated rye No. 133, a plant was found which formed a head-bearing stem, in the upper part of which there were two new aerial nodes with shortened vegetative shoots.

Since special investigations were carried out with this plant from the fall of 1953, its history should be given in detail.

This plant, together with three others, was grown in a large container, with 7 kg of soil, from a seed planted there on September 24, 1953. In the winter 1953/1954 the plant had only one shoot. It spent the winter in an unheated greenhouse. On April 17, 1954, two tiller shoots were found on it. Tillering continued during the spring, and 4 heads were formed on the plant in the summer. Their appearance was noted from June 5 to June 10. During an analysis carried out on July 20, these heads had ripe seeds and were in the state of drying out.

The stems with heads were then cut, and on the plant were left two shoots in the boot stage and 19 shoots in the tiller stage.

The pot with plants remained in the greenhouse until November 30, 1954, when it was found that it had a stem bearing a head, and simultaneously had 2 aerial nodes of branching.

The general appearance of the plant with the indicated stem and the tip of the shoot are shown separately in photographs (Figure 2 and 3) and in the diagram representing its exact copy (Figure 4).

The stem mentioned developed from a shoot, which at the time the stems of the first generation were cut, on July 20 was at the boot stage. It was 50 cm high, and had 7 internodes. The normally formed head was 8 cm long, and contained 18 spikelets. There were no seeds in the head, evidently because the formation of generative organs took place in the autumn during shortened days. Besides the stem mentioned, the plant had 109 living shortened vegetative shoots.

As can be seen from the photograph and the diagram, 2 new nodes were formed in the 2 upper internodes, directly under the base of the spike and lower by 1 whorl, forming typical shortened vegetative shoots.

The shoot of the upper node had five leaves, and that of the second node, seven leaves. Most of the leaves were dark green in color, having excellent turgidity and a healthy, fresh appearance. Both shoots were at once separated from the stem and planted in pots. Later, the pots were placed in the south window of a warm room, and were given additional electric light in the middle of the winter. Unfortunately, one plant died during the winter, and only the plant arising from the upper node was developed.

The temperature conditions of the greenhouse in which this plant was grown until its transfer into the room were characterized by the data given in Table 4.

From the data of Table 4, it can be seen that the number of days with lowered temperature during the autumn period was not sufficiently significant to induce vernalization of "winter" plants. Plants that were "semi-winter" could have been vernalized.

During the growth of plants in the room, the average daily temperatures were not lower than 14°, and the minimum temperatures fluctuated within 10-13°. Therefore, vernalization could not take place during this period. After the onset of stable warm weather on May 3, 1955, the plant was transplanted into the soil. Its behavior in the course of the summer was exactly the same as that of analogous plants in the first experiment. It had an intense process of tillering and all the resulting shoots were closely pressed to the soil surface, had closely-spaced nodes, and were not in any way distinguished from the shoots of the usual plants of perennial rye planted in the spring on adjacent plots. During mass heading of control plants, which had both reproductive and elongated vegetative shoots, our plant was photographed on June 1 (Figure 5), and the number of the living tiller shoots was counted and found to be 33. Later, with the onset of hot weather, tillering ceased and the existing shoots began to die out. During the analysis on July 22, when the seeds in the control were altogether ripe, the plant mentioned had three living shortened vegetative shoots.

To draw a general conclusion from Experiment II, it can be said that it gave a result analogous to that obtained in Experiment I. Considering, however, that the new shoots here arose on such a part of the reproductive stem that its vernalization is beyond doubt, one may confidently conclude that in this case the vernalization changes turned out to be removed.

Experiment III. In the autumn of 1954, the shoots forming on the tips of elongated vegetative stems, and the normal shoots arising from the buds at the lower nodes of the same stem were simultaneously planted in the soil. All of them wintered under natural conditions.

The results of analyses of plants grown from these two types of shoots, carried out June 17, 1955, are given in Table 5, and the external appearance of the plants is shown in Figure 6.

The data of Table 5 indicate that there are very considerable differences, according to all the indicators, between plants grown from the normal sprouting shoots and those from the shoots formed on the tips of elongated vegetative stems.

Experiment IV. On August 31, 1955, the tips of elongated vegetative stems were cut and planted into flats of soil; at the same time, seeds obtained from the same plants were planted.

TABLE 5

Results of Analyses of Anatolian Rye Plants

Origin of shoots	Water content in leaves, percent		Leaf area, cm^2		Transpiration rate, g/m^2 hour		Chlorophyll content in leaves of all whorls, mg/g wet wt.	Average number of shoots per plant					
	Third whorl	Fourth whorl	Third whorl	Fourth whorl	Third whorl	Fourth whorl		Average height, cm	with head	in boot stage	vegetative: elongated	vegetative: shortened	In all
Normal shoots	63.8	66.9	4.3	5.2	114	130	1.414	90	1.9	4.3	4.5	10.1	20.8
Shoots from nodes at the tip of the stem	44.4	49.1	1.1	2.4	87	96	0.717	57	0.2	8.5	14.5	24.8	48.0

TABLE 6

Development of Anatolian Rye Plants

Vernalization treatment	Origin of plants	Number of plants	% of plants having elongated stems	% of plants having only shortened shoots	Height of elongated stems, cm	Water content, percent	Respiration rate, mg CO_2 per g dry wt. for 24 hours
Vernalized	From the tip of elongated vegetative stem	18	100	0	108	57.6	21.3
	From seeds	41	100	0	120	66.3	26.2
Nonvernalized	From the tip of elongated vegetative stem	10	83	17	—	—	—
	From seeds	19	80	20	—	—	—

One flat was transferred to the room prior to the onset of frost, two others were brought there on December 9 and January 9, and the fourth flat was left in an unheated greenhouse. Therefore, the plants in the three latter flats were vernalized for different periods of time. On April 16, 1956, all plants were transplanted into the soil.

Since no differences were observed either in the time of head formation or in the percentage of head-forming plants with various times of transfer of flats into the room, to economize on space we give only data of the analysis of the two extreme treatments (Table 6).

Table 6 shows that the Anatolian rye taken for the experiment represents a population consisting of approximately 20% "winter" forms and 80% "spring" forms. This circumstance complicates the analysis of the results, but does not remove their clarity and conclusiveness. They speak for the fact that, in the "winter" forms of Anatolian rye plants, developing from the tip of elongated vegetative stems, the elongated stems are formed only after vernalization of the shoots which give rise to them, i.e., they are "winter" to the same degree as the shoots formed from the seeds. In other words, the vernalized changes in the meristem which gave rise to them turn out to be completely removed. At the same time, it can be seen from the last three columns of Table 6 that such plants show signs of aging, expressed in the decreased water content and the decreased respiration rate of leaf blades of the same age as well as in the decreased height of the newly forming elongated stems.

What do these facts indicate? They indicate that the meristem which had passed through all vernalizing changes does not stay "spring" in the "winter" perennial cereals, but may, under some conditions, lose its changes

and again require vernalization, without which it is incapable of further development. For this process of changing of its properties, we use the term "removal."

The concept of "removal" first obtained a deep dialectic sense in Hegel, who wrote that "removed is something which is at the same time preserved, which only lost its independence, but which is by no means destroyed because of that" [14]. V. I. Lenin, summarizing the "science of logic" expressed the main essence of this thought in an extremely clear formulation. He wrote: "to remove = to finish (simultaneously to observe) = to preserve" (V. I. Lenin Philosophical Notebooks, Gospolitizdat, 1938, page 107). It is particularly in this sense that we use the term.

The data characterizing the state of plants grown from the tip of elongated stems (Tables 5, 6) indicate that the removal of vernalization changes does not at all represent the return of the plant to its original state. Evidently, in such a plant the age changes, and possibly other changes, are preserved. The removal of vernalization changes represents the process of the graded forward movement of plant development, and not a reverse flow of the vernalization process.

What, then, can be the explanation that a removal of the vernalization changes took place in the experiments described above, while in the experiments of Lysenko [1], Pogosian [3], and Skripchinskii [4] with grafting stems of cotton, soybean, wheat, and rice, nothing similar was observed? The reasons for such differences may be dual: 1) in the former grafting experiments, annual plants were used as experimental objects, while perennial plants were used in our new experiments; 2) the former experiments were carried out in the middle of the summer, favoring the transfer of plants to reproduction, while the vegetative shoots obtained in our present experiment arose in the autumn, when conditions prevented reproduction.

Does this phenomenon occur only in the plants studied by us, or is it more widely distributed?

Up to recent times, a hypothesis was in force according to which the sprouting of winter shoots in a number of species of winter cereals was explained by the fact that the shoots arise, as was thought, from meristerms which had not undergone vernalization [1]. This hypothesis was not liable to direct confirmation because of the absence of reliable methods of differentiating vernalized meristems from nonvernalized ones, and because of the difficulties of morphological analysis of the complex nodes of perennial cereals. The facts described allow for another explanation. It is more probable that, during the formation of new buds in the nodes of the winter perennial cereals, the removal of vernalization changes takes place, since it occurs in the elongated rye stems, as a result of which such shoots again turn out to be winter shoots.

Exactly the same takes place also in all trees and bushes of the temperate zones which are capable of the state of "deep dormancy." It is well known that the buds of such trees may start growing only when they are exposed to the action of lowered temperatures during the winter, but when a shoot is developed from such a bud and new buds are formed on this shoot, in the course of the following summer, then each bud of this kind itself requires the action of lower temperature for its further growth. This may be explained by the changes that took place in the course of the "maternal bud" in the course of the preceding winter, which was then removed during the period of formation of new buds. Analogous processes take place in many bulb, rhizome, and root crop plants.

The biological significance of the removal of vernalization changes in perennial grasses and analogous changes in tree buds has a clearly-expressed adaptive nature, and ensures their successful wintering. If the removal of vernalization changes in the shoots of perennial cereals had not taken place, they would have developed into a boot stage, and would have perished with the onset of winter. In the same way, if the newly formed buds of trees did not enter a state of "deep dormancy," which could only be broken by low winter temperature, they would have started growing in the fall, and would then have been frozen.

It is obvious that natural selection has developed in such plants a special physiological mechanism of removal, which determines the possibility of their repeated adaptation to wintering.

SUMMARY

1. In perennial rye plants, the vernalization changes arising in the meristem may be removed during the formation of vegetative shoots forming from both the apical growing point of an elongated vegetative stem (Anatolian rye, timothy), and from the lateral meristem in axils of upper leaves of a reproductive stem.

2. The removal of vernalization changes helps the plant to form organs which have the ability to undergo hardening, and by this to acquire a great degree of frost resistance.

3. The removal of vernalization changes does not mean rejuvenation, and the plant developing again during this process remains older in age. The process of removal represents one of lengthening the graded development of the perennial plant, but not a return to its original state.

4. The phenomenon of removal of vernalization and similar changes is distributed among all living forms of perennial plants – grasses, shrubs, and trees; because of this, we can hardly be mistaken if we say that it is one of the cardinal physiological processes which determine the ability of these plants to survive the unfavorable period of winter, and in essence determines the possibility of survival of many of them as perennials.

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BRIEF COMMUNICATIONS

WATER UPTAKE BY PLANT ROOTS DURING THE PERIOD OF SAP MOVEMENT IN THE SPRING

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The vital activity of root systems and the beginning of sap movement in many woody plants start very early in the spring, almost simultaneously with the beginning of the melting of the snow or soon thereafter. In grapes, sap movement begins somewhat later, when the soil temperature in the zone of the absorbing roots reaches 8-9° for variety vinifer, 7-8° for the American species Vitis riparia, V. labrusca, etc., and 4.5-5.2° for Amoor grape [2], but still much earlier than the time of bud opening.

There are no direct data in the literature about the way in which water is absorbed from the soil by roots during the period of spring sap movement; however, one may form certain ideas about this from indirect data.

In a number of papers, it has been shown that the soil water may be absorbed only by the root hairs or by the whole surface of white, growing rootlets which have primary root structure [1-7]. According to Muromtsev [3], the primary rootlets of apple live and function for about 12-20 days, after which they develop secondary structure and their function changes. On the basis of the above reports, Budagovskii [4] concluded that for the normal life of plants there should be a continuous process of renewal of the absorbing parts of the roots in the summer. The same opinion exists in relation to grapes [1, 5], but at the same time it was pointed out that the small rootlets in grape plants either drop off or rot in the autumn, like leaves [5], or remain unsuberized and perish in large numbers during the winter [1]. Only the end portions of the thicker roots, which become suberized for the winter, are preserved. In the spring, the growth of these roots continues, as does the formation of of new, small, absorbing rootlets. From this, it follows that after the soil thaws in the spring there are no young absorbing rootlets with root hairs on the roots, which would be absorbing the moisture from the soil. How, then, does sap movement take place in this case?

Kolesnikov [8] writes that the spring sap movement is due to the storage of moisture in the roots; however, this is not very probable, since sap movement is very weak or does not occur at all when moisture is lacking the the soil during the spring, while, with a high soil moisture, one grape vine may form, during the period of sap movement, from 1.4 to 5 or even to 10 liters of sap, flowing under a pressure of 1.5 atmospheres [1]. It is clear that such an amount of water cannot be stored in the relatively small root system of the vine. One is then left to believe that the rootlets are formed in the spring even prior to sap movement. According to Kroemer [9], they appear at the beginning of the flow, with the onset of warm weather; however, Barnard [10] considers that rootlet growth begins only during the period of bud opening.

Merzhanian [1] does not express any definite opinion about this, but the experiment he described, bringing pots of wintering grape vines from cold buildings into a warm one, has shown that as soon as the soil temperature in the pots was raised to 8°C the flow occurred at once; with cooling, the flow rapidly stopped. This indicates, first, that the young rootlets are not yet formed towards the beginning of the flow; second, that the absorption of water by the roots occurs not only through the root hairs and white, thin rootlets, but also through the surface of the older root tips; and finally, that the fibrous rootlets of the root do not die during the autumn-winter period, but are preserved.

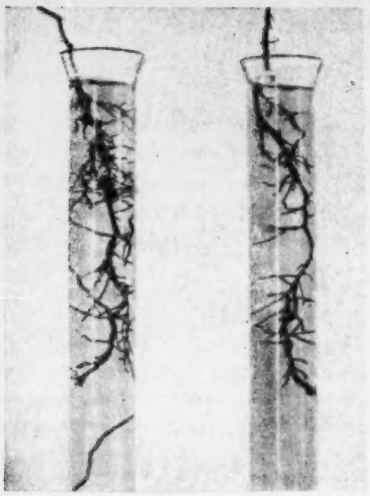

Fig. 1.

Insofar as the second hypothesis is concerned, there are papers which point out the possibility of such moisture absorption. Thus, Rogers [11] has shown that there are autumn and spring maxima in root growth, and that root formation takes place slowly or is altogether absent in the summer in more or less dry soil. How soil absorption takes place at this time can be seen from the experiments of Zgurovskaia and Tsel'niker [12]. They have shown that the roots enter a state of dormancy during the long drought. During this dormant state, the primary bark does not die out but unsuberized passage cells are left among the suberized exoderm, which to a certain degree insure the function of absorbing at the slightest provocation during the drought period. The authors note that such a state of the roots reminds one of that of the cells of growing tissues during their transfer into the state of winter dormancy [13, 14]. One may suppose that the water absorption from the soil by the roots during the winter and spring takes place precisely through these passage cells.

To check this hypothesis, in the spring of 1955 we dug out a number of roots of various grape varieties. The roots were dug out with the soil, without breaking their connections, and were very carefully washed off. The small rootlets were studied under the microscope. It turned out that the majority of the small rootlets were living, and that their tips had a yellow-brown color (as in the experiment of Zgurovskaia and Tsel'niker [12]). The fibrous roots were in the best condition in European Amoor hybrid No. 4 – in the upper layer of the soil down to 15 cm there was only a small amount of dead rootlets. In varieties and selected seedlings of Vinifer, injury of the fibrous roots was more significant to the depth of 20 cm, while in varieties of the species labrusca the injury was more intermediate in nature. In general, in the winter of 1954/1955 the fibrous root system was well preserved, which was not the case in 1953/1954, when the winters were more severe. The data of observations of the force of the flow in these years are in correspondence with this.

A particularly abundant flow occurred in 1955. This indicates that the small fibrous roots die in the autum-winter period, not because the plants shed them as they do leaves, but only as a result of their injury by frost. The observations show that the greater the injury of fibrous roots, the weaker is the spring flow of the grapes. The digging out and the washing of soil from roots have also shown that neither prior to the onset of the flow, nor during the period of the flow itself, was any new formation of absorbing rootlets observed. Consequently, the absorption of moisture during the spring is carried out by rootlets which were formed in the previous year. To determine by which part of the rootlet the water is absorbed, we took roots 2-3 mm thick, with completely intact fibrous rootlets and placed them in test tubes with water, leaving the upper section of the roots emerging from the water for 1-1.5 cm. In one group of roots, the tip 4-5 mm of the rootlets was cut off with a razor blade, while those of the other group were not cut. Observations were carried out on experimental and control roots for 10 days. It turned out that in the test tubes in which the rootlets were uninjured, moisture was secreted from the upper sections of the roots all the time during observations, while the root sections with the rootlet tips cut remained dry. From this, it follows that the moisture is absorbed by the tips of the fibrous rootlets.

The further digging out of roots has shown that the tips of rootlets may, in the spring, continue their growth which was retarded in the previous year. The renewal of root growth in general takes place according to the outline given by Kroemer [9] and Barnard [10]. The tip of the thick rootlet begins to elongate as an effect of cell division in the growing point, and a new absorbing zone appears. The white tip is thicker than the rootlet of the previous year. The same behavior occurs also on the thin branches, with the difference only, that their tips do not thicken. If the small rootlet died out, then one or several branches appeared at its base. In 1955, we dug out at the same time the roots of a number of woody plants – elm, maple, oak and other species growing under forest conditions, and also apple trees in the garden, and found that the richest fibrous root system at the very surface of the soil had been preserved completely; immediately with the onset of warm weather, the sprouting of young rootlets had begun, continuing the growth of the rootlets of the previous year.

This should have been expected, because the autumn growth of the roots [8] is a preparation for the beginning period, and the absorption of water by the old rootlets through passage cells is retarded as compared to its absorption by young roots. It is difficult to believe that in the course of autumn and winter the plant would lose its richest fibrous root system, only to create it anew in the spring, when it must develop a not-less-extensive leaf system.

Measurements of soil temperature in the root zone and observation of the beginning of sap flow and formation of new roots in 1955 have shown that the flow begins in the European-Amoor grape hybrid at 4-4.5° (No. 4 and No. 82), at 5-5.5° in hybrids of vinifera X labrusca (Nagrada, No. 52, No. 17, Russian Concord), and at 6-6.5° in varieties of vinifera (Tambovskii pink, Tambovskii green, Seianets Malengra, etc.), which is somewhat lower than certain data existing in the literature. The rate of flow increases with a rise of the temperature above the indicated minima.

The formation of new roots is observed at a higher soil temperature – at 8-10° in European-Amoor hybrids and wild Amoor, at 10-12° in hybrids of vinifera X labrusca, and at 12-14° in varieties of vinifera. The beginning of formation of new rootlets in all varieties coincided with beginning of bud opening. It is obvious that if this did not occur, the absorption of moisture merely by the tips of last year's rootlets would not have been able to satisfy the vine's water requirement, at a time when the processes of active shoot growth and the growth of the enormous transpiring leaf surface are taking place.

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WINTER DEVELOPMENT OF BUDS

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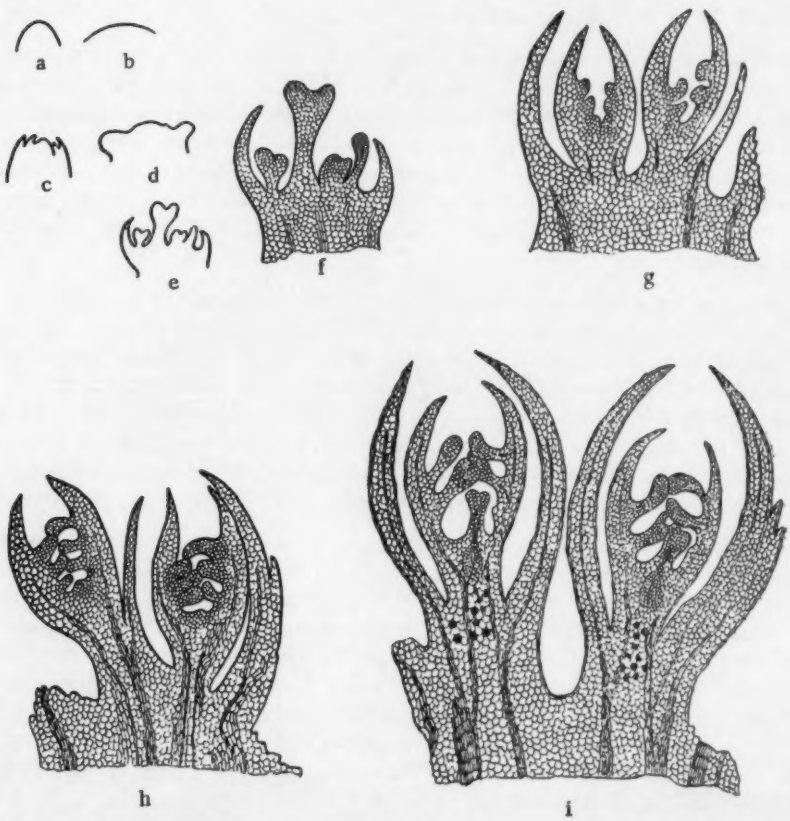
The question of the state of buds during the winter period is at present regarded with a number of different points of view. Zheleznov [1], Viktorov [2], Riadnova [3], and Sergeev [4] have observed a number of morphological changes in fruit buds in the winter time. Askenasy [5], Ro [6], Minina [7] consider that no morphological changes take place in fruit buds, although Ro [6] had observed the phenomenon of winter growth in one of the warm winters. Lately, this question was studied by Petrovskaia [8, 9], who made cytological observations on fruit buds in a number of woody and shrubby plants under conditions of the Moscow Region. She found no winter growth in the objects studied, and on the basis of her data, drew a general conclusion about the absence of winter growth, without reservations concerning the geographic location and the plants to which this conclusion could apply.



The state of apple flower buds (significance of letter designations is given in the text).

The state of buds during the winter is of great significance, since their frost resistance is connected with the degree of flower differentiation. It is because of this that the question of winter growth continues to attract the attention of investigators.

The author of the present paper did not study specifically the question of the winter development of buds, and the material described here has been obtained in passing in the course of other investigations. Since no single opinion exists concerning the question, we consider that our data may be of interest, all the more because they were obtained under the specific conditions of the Primorskii region.

The investigations were carried out from July 1949 to March 1950. During this period anatomical investigations of fruit buds, including drawings of the buds, were carried out monthly. In the present paper, only a part of the drawings are given, while descriptions are given according to the periods in which investigations were made. The outlines of buds, arbitrarily magnified, are given in Figures a, b, c, d, e, f while buds are presented in Figures g, h, and i at a magnification of 320 ×.

During the first investigation (July 14), fruit and leaf buds were taken. At this time, both types of buds were represented by small protuberances of meristematic tissue. The differences between them consisted only in the fact that in fruit buds the growing point was slightly convex and had a wide base, while leaf buds had a more convex growth point and a narrow base (Figures a, b).

A month later (August 15), at the time of the following examination, noticeable changes had taken place in buds. In leaf buds, the leaves were initiated, in the fruit buds, protuberances of flower buds were present (Figures c, d). In some of the fruit buds even more noticeable changes had taken place – the flower buds became separate and tissue differentiation became noticeable, as can be seen in Figures e and f, where drawings of the same bud are given at different magnifications. Meristematic tissue, composed of small cells characteristic of the growing point, remained only in the upper part of the flower bud. The cells in the middle region of the bud and at its base increased in size. In addition, rows of provascular cells were formed at the base of the bud, indicating the beginning of development of the conducting system.

In the following month and a half (August 15-October 1), very great changes took place (Figure g). During this period, the meristematic proturberances of the stamens had arisen inside the flower buds, and the sepals and petals, in which the process of tissue differentiation was already expressed, had been formed. The cells of the basic tissue had greatly increased in comparison with the cells of meristematic protuberances. The first provascular cells were formed in the lower part of petals. The whole surface of both petals and sepals was covered with epidermis, which was not observed during the first examination. In the groups of provascular cells which had been developed earlier, differentiation was increased, and groups of more or less thick-walled cells became clearly visible.

In the course of the period from October 1 to November 1, less noticeable changes were observed. They were expressed only in the increase of the size of stamens and in the appearance of the hardly noticeable protuberance of the future pistil. Thus, in the beginning of November one may distinguish in the flower only sepals, petals and stamens, while the pistil was hardly noticeable. The flower was not formed towards the beginning of the winter period. During November, as in the preceding month, the changes in the buds were small. They were expressed in a slight increase in the size of the bud, and in intensification of tissue differentiation. Provascular cells were formed almost throughout the whole length of the sepals and petals, the differentiation of the conducting system was more noticeable, and the epidermis was more clearly seen (Figure h).

More significant changes took place during December. During this period, the size of the stamens was increased and their form was changed: the filaments of the stamens were increased in length, and thickenings – the future anthers – had been formed on their ends. Tissue differentiation was begun in the stamens themselves. The cells at the base were increased in size, and separate provascular cells of the conducting system had appeared among them. In the receptacle, among cells greatly increased in size under the protuberance of the pistil, an area of small-celled meristematic tissue has appeared. As further investigations have shown, this was the beginning of ovary formation, i.e., the pistil as a whole began to develop from both sides. A meristematic protuberance which had arisen on the surface of the receptable, and the meristematic tissue inside the receptacle, further combined into a single area of meristematic tissue; the styles and stigmas are formed from the upper part, and the ovaries from the lower part.

Noticeable changes took place in January. During this period the volume of the entire bud was significantly increased due to the enlargement of the cells; in the stamens, continuous threads of provascular cells have arisen, which give rise to the conducting system; the style of the pistil increased in size, and the outlines of the future locules were indicated in the ovary.

The last investigation was carried out on March 3. During the preceding February, all parts of the flower were greatly increased (Figure i) – petals, sepals, stamens, pistil. Tissue differentiation became intensified and the conducting system became clearly distinguished. The style of the pistil elongated noticeably, and the formation of the stigma was indicated on its tip. In the ovary, all locules became clearly seen. Towards March, as shown in Figure i, all parts of the flower were already expressed in the fruit buds.

From these investigations, it can be seen that under the conditions of the Primorskii region, the fruit buds of apple undergo continuous morphological changes, beginning with the moment of growing-point formation in July, and continuing throughout the entire subsequent autumn and winter periods.

Whether growth in the winter takes place only on relatively warmer days, or under any conditions, we cannot say on the basis of our data; for an answer to this question, anatomical investigations would have to be carried out considerably more often than once a month. According to the papers of Viktorov [2], Riadnova [3], Sergeev [4], if growth does take place in low sub-zero temperatures, it is very insignificant. In general, bud growth takes place during the warm sunny days. The contradictions in the data obtained by various investigators may, in our opinion, be explained by the fact that investigations were carried out in various climatic zones and with different species. Winter growth of buds in Southern regions was clearly expressed in almost all varieties studied; winter growth of buds in northern regions was noted in some woody varieties and it was absent in others.

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RESPIRATION OF POTATO PLANT TISSUES IN CONNECTION WITH RESISTANCE OF THE PLANT TO TUMOR-PRODUCING AGENTS

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Biochemical studies concerning the nature of the resistance of potatoes to tumors induced by the fungus Synchitrium endobioticum (Schieb.) Pers., are of considerable theoretical as well as practical significance. A knowledge of the factors which determine the resistance of potatoes to tumors would make it possible to scientifically suggest basic methods for a rapid determination of the resistance of numerous varieties of specimens to tumors at all stages of investigation on selection and varieties, and it would also be possible to propose methods for directing changes in the hereditary nature of the potato and control its development with the object of retaining and increasing its resistance to tumors

The few papers concerned with this problem [1-9] do not touch upon a study of the respiratory process which occupies a central position in the metabolism of living cells.

In 1954-1955 we measured the respiration (manometrically in a Warburg apparatus) of tissues from organs in varieties of potato plants resistant to tumors (Grentsmark, Karnea) and in susceptible varieties (Vale, Vol'tman) both in soil free of and soil contaminated with the causal organism. Respiration was measured twice in the leaves, tubers, outgrowths from the tubers, and in the roots during the prolonged vegetative period, and also in the sprouts not infected even on the 4th, 8th and 16th day after inoculation with the tumor-inducing organism.* Exactly 0.3 g of experimental material was placed in the flask, and covered with 5 cm^3 of phosphate buffer (pH-5.3). Alkali (15% solution of KOH) was poured into the central cylinder. Average specimens of experimental tissues were chosen as follows: cylinders 0.7 mm in diameter and 1 cm long were cut with a cork borer from the zone of top eyes in the tuber; from these, sections 0.3-0.5 mm thick were subsequently prepared. These sections were washed frequently with water and were then dried with filter paper. Discs were cut from the leaves with the same cork borer (two from each leaf); these were likewise washed and dried. In the case of sprouts, sections were made from the top part of the shoot (4-10 mm long), and the roots were also cut into pieces 1 cm long. Respiration was measured at 26° for an hour.

The result of respiration measurements on healthy potato sprouts as well as on those 4, 8, and 16 days after inoculation with a tumor-producing agent are given in Table 1. It appeared that in the sprouts of the Grentsmark variety, resistant to tumors, contact with a tumor-producing agent brought about an increase in respiration only during the very first few days after inoculation, later there was a sharp decrease in respiration. At the same time, a stimulation in the respiration of shoots from the susceptible variety Vale was observed after inoculation at all three periods of measurement. These data were somewhat of a surprise from the point of view of existing data concerning the specific protective role of oxidation processes, however they agree with results obtained for the respiration of tubers (Table 2); these agree with the increased respiration during constant contact with tumor-producing agents observed only in tubers of susceptible varieties, and the decreased respiration in tubers from the inoculated plants of the resistant varieties.

*The days were counted from the beginning of the contact of young shoots with pieces of fresh tumor sections which served as sources of inoculation; contact continued 2 days.

TABLE 1

Respiration of Potato Sprouts, in Healthy Potato Sprouts as Compared withThose 4, 8, and 16 Days After Inoculation with a Tumor-Producing Agent (in μl O_2 per gram fresh weight per hour)

Days	Vale		Grentsmark	
	healthy	diseased	healthy	diseased
4	663.18	713.9	402.25	572.56
8	486.04	508.9	282.06	235.5
16	215.02	318.96	270.09	215.42

TABLE 2

Respiration of Tubers and Tubercles (in μl O_2 per 1 g fresh weight per hour)•

Variety	Date of analysis	Tubers				Tubercles	
		healthy		diseased			
		1	2	1	2	1	2
Vale	July 15	85.8	82.2	100.8	109.6	316.2	310.0
	Aug. 10	99.0	93.6	135.6	128.0	289.0	—
Vol'tman	July 20	120.0	112.8	142.8	141.3	240.9	254.0
	Aug. 15	134.8	124.4	167.2	158.4	223.3	—
Grentsmark	Aug. 13	103.2	88.2	90.6	80.6	—	—
	Aug. 8	70.8	63.6	48.0	49.2	—	—
Karnea	July 18	101.6	119.3	67.2	73.2	—	—
	Aug. 13	32.9	37.9	25.2	—	—	—

•Parallel columns (1 and 2) in the table are results from duplicate measurements of respiration.

The rate of respiration in the tubercles was two to three times greater than that in the tubers. However, the rate of respiration in the internal portion of the tubercles was considerably less than that at the surface. For example, during the measurement for respiration on July 29th, 260.5 μl oxygen were utilized at the surface of the tubercles in variety Lorkh during the first measurement, and 257 μl during the second; that used in the inner portion was 151 and 171 μl, respectively.

TABLE 3

Respiration of Leaves and Roots in Healthy and Tumor-Infected Potato Plants (in μl O_2 per 1 g fresh weight per hour)

Variety	Leaves		Roots	
	healthy	infected	healthy	infected
Vale	446.4	380.6	204.0	156.0
Vol'tman	473.5	445.2	262.8	172.6
Grentsmark	621.7	439.2	192.5	61.6
Karnea	436.2	364.2	196.9	171.8

The rate of respiration in the leaves exceeded that of all the remaining potato plant organs, and even that of the tubercles. However, it was always lower in the infected plants than in the resistant ones, as well as in varieties susceptible to tumors. The roots resembled the leaves in that the rate of respiration decreased after infection in all the varieties we studied (Table 3).

It is frequently thought that an increase in respiration occurs regularly as the result of the infection of higher plants with obligate parasites. The literature on this subject has been summarized in several reviews [10-13].

As one can see from the experimental data given above, a stimulation of respiration after the inoculation of potatoes with tumors was observed only in infected tubers of susceptible varieties but most especially in the tissues where the tumors had developed in tubers or inoculated plants of resistant varieties; whereas in the leaves and roots of infected plants of resistant as well as susceptible varieties, the rate of respiration was lower than it was in the corresponding organs of healthy plants.

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FREE AMINO ACIDS IN TEMPORARILY CHILLED WINTER WHEAT

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A study of the amino acid content of various parts of the seed subjected to temporary chilling has been reported in only one paper by Pashevich [1]. The author studied the amino acid content of glutanin and gliadin isolated from the embryo of such seeds of winter wheat. It was shown that the change in the amino acid content of gliadin was negligible, whereas the change in glutanin was quite clear. It was discovered that there was practically a twofold decrease in the content of tryptophan and dicarboxylic amino acids in the proteins of the embryo, as compared with the quantity of these amino acids in dormant seeds. After a temporary chilling, there was a small increase in the quantity of arginine. The sharp decrease in the amount of tryptophan and dicarboxylic acids after chilling was the basis for the author's conclusion that these amino acids are not deeply seated in the protein molecule, but occur on its surface.

During the present study we did not investigate the structure of the proteins, but instead we made a comparative study of the content of free amino acids in the embryo and in the endosperm of winter wheat Moskovskaia 2453 by means of paper chromatography, whereby only qualitative determinations of the amino acids were made.

The extraction of free amino acids was performed according to Dent, Stepka, and Steward's [2], and Allsop's [3] method.

One-gram portions of fresh material were covered with a portion of 80% alcohol 10 times greater than the sample, carefully ground in a mortar, transferred to centrifuge tubes, and were left at room temperature for two hours. After that the suspensions were centrifuged at 2500 rev. per min. The scant volume of alcohol was decanted into a flask. Following this, the specimens were again covered with 2 ml of 80% alcohol, shaken well, and again left for two hours. Such a procedure was performed three to four times. After each centrifuging the alcohol extracts of amino acids were decanted into flasks and evaporated to dryness under vacuum at a temperature no higher than 35°. Each of the dry residues was dissolved in 0.5 ml of 80% alcohol.

The solutions obtained were analyzed for amino acid content by means of one-dimension descending paper chromatography. The determination of amino acids was performed on paper from the Leningrad plant No. 2. This paper gives good chromatograms with a uniform clear background of the solvent and compact spots of amino acids. The paper was first rinsed with distilled water. Sheets of paper 40 x 50 cm were used. Drops of the solution being tested and those from a control solution with the probable amino acid were placed 5 cm apart on the paper.

Two chromatograms of each extract were made simultaneously: one in a solution of water-saturated butanol (80%) and acetic acid (20%), the other in a solution of water-saturated phenol (80%). The time of exposure was 72 hours. The chromatograms were removed from the solvent and dried at room temperature for 2-3 hours; following this, in order to develop the chromatograms, they were sprayed with a 0.2% solution of ninhydrin and dried again in the air at room temperature for 24 hours; this is recommended [4] in order to avoid the partial oxidation of amino acids at high temperatures [4]. The amino acids were identified by matching the coefficient for the rate of movement (R_f) and the color of the spots with spots of the supposed amino acids.

Our investigations disclosed only two free amino acids in the endosperm of the dormant seeds: cystine and aspartic acid (see table).

TABLE

Content of Free Amino Acids in the Endosperm and Embryo at Different Periods of Chilling

Endosperm						Embryo					
dormant seeds	germinating seeds	12 days	24 days	36 days	50 days	dormant seeds	germinating seeds	12 days	24 days	36 days	50 days
cystine, aspartic acid	threonine, tyrosine, aspartic acid, glutamic acid, arginine, histidine	alanine, serine, threonine, aspartic acid, glutamic acid, lysine, histidine, proline	alanine, valine, leucine, glutamic acid, arginine, histidine	valine, methionine, lysine, arginine, histidine, aspartic acid	alanine, threonine, leucine, serine, aspartic acid, lysine, histidine, arginine	valine, serine, threonine, cystine, aspartic acid, glutamic acid, arginine, proline, tryptophan, histidine	alanine, valine, leucine, serine, threonine, cystine, tyrosine, aspartic acid, glutamic acid, proline, arginine, histidine	alanine, valine, leucine, serine, threonine, cystine, tyrosine, aspartic acid, glutamic acid, arginine, histidine, trypto-phan, proline	alanine, valine, leucine serine, threonine, cystine, phenyl-alanine, tyrosine, aspartic acid, glutamic acid, arginine, lysine, histidine, proline	valine, serine, threonine, cystine, aspartic acid, glutamic acid, lysine, histidine, arginine, proline	alanine, valine, leucine, serine, threonine, cystine, methionine, aspartic acid glutamic acid, lysine, histidine, proline

The low content of free amino acids in the endosperm of dormant seeds can be explained by the fact that nitrogenous compounds are present here primarily as proteins. There were quite a few more amino acids disclosed in the embryo of the dormant seed; namely: valine, serine, threonine, cystine, aspartic and glutamic acid, arginine, histidine, proline, tryptophan. This large variety of amino acids agrees with our data [5] concerning the content of amino nitrogen, which we found to be higher in the embryo than in the endosperm.

On the basis of the chromatograms obtained, it may be concluded that the increase in the amino nitrogen content of the embryo occurred not only because of an increase in quantity, but also because of the variety of amino acid composition.

Free amino acids in intact seeds of dormant winter wheat were studied by Kolobkova and Kudriashova [6]. The authors tried to show precisely which acids dissolved in water during germination and passed through the epidermis of the seed, since it is known that some amino acids are substances which inhibit the germination of seeds. The investigation was also carried out using chromatograms. The authors showed that there were nine amino acids in the intact seed of winter wheat Liutestsens (alanine, valine, lysine, leucine, cystine, aspartic and glutamic acids, arginine, tryptophan). It is evident from the data presented that the varieties of winter wheat Liutestsens and Moskovskaia 2453 differ from each other somewhat in their content of free amino acids. It is possible that this is associated with the fact that amino acid composition of the proteins in seeds of the grains varies in the different species and varieties and depends on the conditions of growth and the geographical location of the plants; this has been observed by many authors [7-10].

A study of the chromatograms obtained from extracts of the endosperms and the embryos of germinating seeds revealed that there was a diversity in the amino acid content of one or another organ during this period. An increase in the diversity of amino acids in the endosperm is evidence that there was a dissociation of stored protein molecules and that these dissociated molecules could later diffuse into the embryo, where they were utilized in the synthesis of protein. During enzymatic cleavage of the stored proteins in the endosperm, which accompanied germination, the dicarboxylic acids – glutamic and aspartic (which as we know, are capable of engaging in the complex system of reactions occurring continually in the living cell) were split off along with threonine, arginine, tyrosine and histidine. After they had combined with a molecule of ammonia, these amino acids then yielded amides (asparagine and glutamine) which are the basic initial compounds in the synthesis of protein.

The appearance of new amino acids – leucine, tyrosine, alanine – was also observed in the embryo of germinating seeds; alanine, according to Khristeva [11], can have a stimulating effect on the growth of plants, activating one or another of the enzymes.

The increase in the amino acid content of the embryo may occur as the result of their diffusion from the endosperm; however, their formation during hydrolysis of proteins in the embryo itself should not be excluded. It should be noted that when phenol was used as the solvent during the development of the amino acids two spots of aspartic acid appeared. The same phenemenon was observed by Dent, Stepka, and Steward [2] who studied the free amino acids in root tips and stamens of Tradescantia during various stages of growth using the chromatographic method. This phenomenon has not yet been explained in the literature. It is possible that there are two isomers of aspartic acid.

During intermittent chilling there was a further change in amino acid content, as the data from the chromatograms show. At that time, new amino acids appeared in each succeeding sample taken. Some of the acids which had appeared during the previous period, disappeared (see table). For example: if we compare those acids which appeared in the endosperm on the 24th day of chilling with those which appeared on the 12th day, we see that valine, arginine and leucine were present, whereas serine, lysine, aspartic acid, proline, and threonine had disappeared. Threonine and phenylalanine, which were lacking on the 12th day, appeared in the embryo on the 24th day, and tryptophan disappeared. The same sort of thing was also observed during the succeeding periods of chilling. These data are evidence that during chilling some amino acids are regularly involved in metabolism, and others are freed; they are also evidence that at this time there is a profound transformation in the proteins and their components, the amino acids.

If one compares the amino acid content of the endosperm with that of the embryo, one can see that there were more free amino acids in the embryo of the dormant seed, as well as in all the periods during chilling, than

there were in the endosperm. This is in complete agreement with the data concerning the amino nitrogen content; there was considerably more in the embryo than in the endosperm.

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BIOSYNTHESIS OF MORPHINE IN THE OPIUM POPPY AND ITS MOVEMENT IN THE PLANT

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The biosynthesis of morphine, the principal alkaloid in the opium poppy (Papaver somniferum), has not yet been investigated. There is some data in the literature concerned chiefly with the amount of morphine in the milky sap of the various organs of the poppy. The works of Emde [1, 2] and Robinson [3] in which the possible paths of morphine biosynthesis were discussed are completely theoretical, and the hypotheses expressed are not supported by experimental data. The object of this present investigation was, above all, to explain the possibility of morphine synthesis from some kind of amino acid; this has been demonstrated for several of the other alkaloids [4, 5, 6].

Young poppy plants (in the branching stage) grown in garden beds were used in the experiment. Before the experiment the plants were dug up, weighed, and infiltrated through the leaves with a 0.1% solution of amino acids in a vacuum desiccator. After the infiltration the plants were weighed again and placed in water for 24 hours, fixed in Koch's solution, weighed, and analyzed for morphine using Soboleva's micromethod [7].

TABLE 1

Morphine Content of Plants Infiltrated with Solutions of Amino Acids (in mg per 20 plants)

Amino acids	Amino acid content in mg	Aerial part	Roots	Total	Amino acids	Amino acid content in mg	Aerial part	Roots	Total
	Experiment I					Experiment II			
Lysine	28.8	1.280	0.204	1.484	Lysine	27.2	1.220	0.195	1.417
Glycine	23.8	0.900	0.178	1.078	Glycine	28.0	0.880	0.160	1.040
Glutamic acid	28.2	0.880	0.188	1.068	Glutamic acid	28.1	0.860	0.196	1.056
Water	—	1.060	0.206	1.266	Water	—	1.070	0.200	1.270

The results (Table 1) show that the infiltration of glycine and glutamic acid not only did not promote the accumulation of morphine, but actually brought about somewhat of a decrease in alkaloid content as compared with the control. Apparently one can conclude that these amino acids do not have a role in the biosynthesis of morphine. In fact, it has been shown by Kuzina and Merenova [8] that when radioactive glycine was introduced into the poppy stem the coefficient of C^{14} utilization in the formation of morphine was extremely small (0.024-0.30%). It was a completely different matter with lysine; in our experiment the infiltration of lysine brought about a 20% increase in the morphine content of the leaves as compared with the control. It is true that such an effect was not observed in the roots; during the short period of the experiment, lysine apparently, did not move to the roots from the leaves in a quantity large enough to have an effect on morphine accumulation. The results obtained led us to conclude that lysine can be one of the initial products in the biosynthesis of morphine.

In order to confirm this conclusion the following experiment was set up: a pipette (both ends drawn into a capillary) filled with a 0.1% solution of lysine was inserted into the stem of a poppy plant in the budding stage growing in the garden. The experiment was set up in triplicate with ten plants in each replicate, and continued for seven days; the control plants were injected with water.

TABLE 2

Accumulation of Morphine Following the Injection of a Lysine Solution into the Poppy Stem

Plant organs	Absolute dry wt. in g			Accumulation of Morphine in mg		
	before the experiment	after the experiment		before the experiment	after the experiment	
		control	expt.		control	expt.
Primary bud	0.56	2.87	3.06	0.58	3.54	4.83
Lateral buds	0.20	2.04	2.42	0.24	2.65	3.82
Leaves	10.60	16.75	17.77	5.46	8.06	7.83
Stem	3.90	16.0	17.50	1.56	5.60	5.43
Root	1.02	4.72	5.39	0.46	1.89	2.26
Total	16.28	42.38	46.14	8.30	21.74	24.17

The data obtained (Table 2) support the conclusion concerning the participation of lysine in morphine synthesis. The total amount of morphine in the experimental plants increased, however it did not increase in all the organs. For example, the increase of morphine in the buds as compared with the control was 36-44%, in the roots – 19%, but in the leaves and stem the amount of alkaloid remained practically unchanged. This phenomenon might be explained thus, the lysine injected into the plant, together with other building materials, moved into the most rapidly growing organs where morphine synthesis occurs. Actually, after comparing the rate of growth of the various organs and the intensity of mrophine accumulation in them (Table 3), we found a distinct relationship between these values; the most rapidly growing lateral buds of the experimental as well as the control plants accumulated morphine more intensively, the somewhat slower growing primary bud was next, then the roots, the stem, and finally the leaves with the vary slowest rate of growth during this period.

TABLE 3

The Rate of Growth of Certain Organs in Poppy and the Intensity of Morphine Accumulation in Them During 7 Days

Plant organs	Rate of growth in g per 1 g dry weight		Intensity of morphine accumulation in mg per g dry weight	
	control	expt.	control	expt.
Primary bud	1.35	1.38	1.73	2.34
Lateral buds	1.64	1.82	2.15	2.93
Leaves	0.45	0.50	0.19	0.17
Stem	1.21	1.27	0 41	0.36
Root	1.49	1.36	0.49	0.53

However, this phenomenon can be explained by the increased movement of materials from which morphine is synthesized (lysine for example) to the most rapidly growing organs, as well as the movement of the alkaloid itself from the slower growing organs. In connection with this our next problem was to clarify the possibility of morphine translocation throughout the plant. The daily variation in morphine content of the poppy

leaves was studied first. In order to do this, we chose 200 plants as nearly alike as possible growing in the garden, and made leaf sections of a specific area after 4, 9, and 19 hours from leaves in the ninth layer, those in the 15th layer, and from leaves on the first lateral shoot.

We observed an increase in the morphine content of the leaves during the period between the 4th and 9th hours, and then a decrease by the 19th hour (Table 4).

At the same time the greatest fluctuation in morphine content was observed in leaves from the fifteenth layer on the primary shoot and in the leaves of the secondary shoot. Evidently there was a very intense synthesis of the alkaloid in the young actively growing leaves; a considerable quantity of the alkaloid synthesized moved to the developing buds and roots which were still growing.

A test for the amount of morphine in the milky sap which flowed from the cut flower stalk on the main shoot revealed that the latex became richer in morphine after 9 hours, i. e., when there was maximum movement of the alkaloid from the leaves (Table 5). Apparently morphine is translocated in the latex system of the plant.

TABLE 4

Amount of Morphine in mg per 1000 cm^2 Leaf Area at Various Times of the Day

Leaf layer, counting from the bottom	4 hours	9 hours	19 hours
Ninth	3.29	4.98	4.07
Fifteenth	3.99	7.70	4.57
Leaves from secondary shoot	2.83	4.85	3.08

TABLE 5

Morphine Content of the Milky Sap of Poppy

Time in hours	Absolute dry wt. in mg of latex escaped in 30 min.	Morphine content of the latex in %	Loss of morphine in mg after 30 min.
4	0.0702	6.7	4.7
9	0.1279	5.5	7.0
12	0.1511	5.8	8.8
16	0.1935	5.5	10.6
20	0.1760	4.8	8.5

The increased accumulation of morphine in the buds and roots is correlated with their very rapid growth; this results in an increased movement into them of materials from which morphine is synthesized, as well as of morphine itself. Therefore, when lysine was introduced into the plant stem we did not observe any increase in the amount of morphine in the stem and leaves, the organs which grow slower than the buds and roots.

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BREAKING THE DORMANCY OF RECENTLY HARVESTED POTATO TUBERS BY XANTHOGENATES

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The dormancy of recently harvested potato tubers can easily be broken when they are treated with sulfhydryl substances, or else compounds which are capable of enolizing the thioketone group into the sulfhydryl group [1-5]. It is also known that ethylene chlorohydrin, which does not have a sulfhydryl group, increases the content of glutahione.

The stimulating effect of xanthogenates, sulfhydryl compounds with the general formula $R \cdot O \cdot C(=S)-SH$ has been established [6]; these compounds stimulated the growth of the root system in wheat and rape when the seeds were treated with concentrations of these compounds ranging from 1 : 1000 to 1 : 50,000 before the seeds were planted. When homologous xanthogenates were studied, there was no difference observed in their stimulating effect on growth. Sulfhydryl compounds (cystine, glutathione, thioglycolic acid, etc.) activate the oxidation of S = S enzymes (papain, some dehydrogenases, amylase, etc.).

An increase in the proteolysis of proteins (a process which leads to the accumulation of SH-amino acids) is especially important for growth; this process is activated by autocatalytic disulfide enzymes. An accumulation of amino acids produces a situation conducive to the resynthesis of proteins in newly developing tissues and organs; in the long run this makes conditions favorable for a change from the dormant state to that of active growth. Assuming this possibility, and also keeping in mind the investigations concerning the removal of dormancy in tubers by treatment with ammonium thiocyanate and thiourea [1,5], we decided to study the effect of xanthogenate salts on the breaking of dormancy. In contrast to thiourea and ammonium thiocyanate, where the SH-group can be formed during the enolization process, a study of the xanthogenates is of even greater interest since they have a stable SH-group.

Treatment with sulfhydryl compounds produces an intermittent depression of respiration as well as the activation of some oxidizing enzymes – phenoloxidase [7], peroxidase [8], and catalase [9]. However, the appearance of intermittent anaerobiosis is, as we know, accompanied by an increase in hydrolytic processes, and also an increase in the activity of dehydrogenases [10, 11]. In all probability, there is also a gradual restoration in the activity of metal-containing oxidases resynthesized at the expense of energy from dehydrogenation processes at the same time.

The experiments concerning the breaking of dormancy in potato tubers were done with the varieties Lorkh, Berlikhingen, and Epron. A month after harvest Lorkh tubers (harvested September 18, 1956) were treated for two hours with concentrations of ethyl xanthogenate (EXG) ranging from 0.03-0.1%.

In order to get a better penetration of the solutions into the tissues, the tubers were cut in half before the treatment. Cut tubers treated for two hours with either water or a 2% solution of thiourea (TU) served as controls. After the treatment the liquid was decanted and the tubers were left out in the air for 12-15 hours. On the following day the tubers were rinsed with distilled water, dried in the air a bit, and planted in pots in the green-

house. As soon as the sprouts appeared the plants were supplied with adequate illumination from luminescent DC lamps. Tubers treated with a 0.1% concentration of EXG began to sprout on October 28, 1956; the sprouting was uniform. In the remaining variants, sprouting occurred later and was not uniform. The plants were measured 32 days after planting. The data are given in the table and figure.

The data given in the table show that EXG hastened sprouting and growth in tubers which had emerged from dormancy. Tubers treated with EXG began to sprout somewhat earlier than tubers treated with water and TU; they sprouted more uniformly and in the first month the plants had surpassed the control plants and those from tubers treated with thiourea in the growth and development of the aerial parts. It should be noted that the stimulating effect of EXG on recently harvested tubers was generally greater.

In the experiment described, the tubers had been kept in a warm place for a month after harvesting and were not fully dormant, therefore in order to break the dormancy it was necessary to use a weaker concentration of TU since a 2% solution had some harmful side effect and brought about formative changes of the leaves.

The sprouting of the tubers treated with water could also be explained by the fact that they were in a state of forced dormancy. The experiment ended when the plants from the tubers treated with EXG and TU began to bloom in the beginning of December. By this time the differences in growth of these two variants were no longer discernible.

In the summer of 1957 the experiment was repeated with freshly harvested tubers of the Epron variety. They were harvested July 5th. The tubers received the same treatments as those in the preceding experiment. The concentration of EXG was increased to 0.5% in view of the fact that freshly harvested tubers of this variety are fully dormant. Tubers treated with water, and those with a 2% solution of thiourea served as controls. After the tubers had been treated for two hours, wilted, rinsed, and dried they were planted in pots on July 6th. Tubers treated with EXG and TU began to sprout on July 26th, the controls on August 17th. By September 1st, 60% of the tubers treated with EXG and 80% of those treated with TU had sprouted. By this time only 10% of those treated with water had sprouted.

Figure. The effect of EXG and TU on the development of potato plants. 1) Control (water); 2) plants from tubers treated with EXG; 3) plants from tubers treated with TU.

The plants were harvested on October 2nd after 66 days of growth (from the first appearance of the sprouts). The yield of tubers per plant obtained from tubers treated with EXG was 62 g, from tubers treated with TU 26.2 g, from the controls 5 g.

From the second experiment one can conclude that a 0.5% concentration of EXG stimulated the sprouting of freshly harvested tubers; plants from the stimulated tubers considerably surpassed the plants from the control tubers both in growth and tuber formation. The difference between the plants obtained from tubers treated with TU was observed only at the time at which tubers began to develop; they began to develop earlier in the plants from tubers treated with EXG. One can conclude that the concentration of EXG (0.5%) was somewhat too high and even toxic for the freshly harvested tubers since only 60% of the tubers sprouted and the remaining ones exibited somewhat of an inhibition to sprouting. In this experiment no formative changes were observed

in the leaves after treatment of freshly harvested tubers with TU. It should be noted that all the eyes sprouted in the tubers treated with TU, whereas apical dominance was not removed with EXG.

TABLE

The Effect of Ethylxanthogenate on Breaking Dormancy in Potato Tubers (data from October 18, 1956)

Treatment of tubers	Date of sprouting	Total sprouted on Nov. 20 in %	Average height of plants in cm
Water (water control)	10/31	80	24.2
Thiourea, 2%	10/30	100	24.2
Ethylxanthogenate, 0.1%	10/28	100	40.8

The third experiment was with freshly harvested potato tubers, variety Berlikhingen, which were fully dormant. The tubers were treated with a 0.5% solution of EXG, a 2% solution of TU, and water for three hours. After wilting and drying, the tubers were planted in pots in the greenhouse on September 10th. Tubers treated with TU and EXG began to sprout on October 7th. The control tubers did not sprout.

EXG and TU stimulated the sprouting of tubers while the controls remained fully dormant throughout the experiment. The inadequate illumination during the fall resulted in a somewhat constricted leaf area and excessive growth of the internodes; these characteristics were even more prominent in the plants from tubers treated with TU.

These experiments indicate that ethylxanthogenate can be considered as a new growth stimulator of potato tubers.

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THE UTILIZATION OF POTASSIUM β-NAPHTHOXYACETATE AND 2,4-DICHLOROPHENOXY-α-BUTYRIC ACID IN VITICULTURE

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The number of methods developed in which chemical regulators are used in plant culture is obviously inadequate. Furthermore, the utilization of growth regulators can play a significant role in increasing the yield of many agricultural crops [1-4]. At the present time some of these chemicals are used on a large scale only as herbicides [5,6].

Some investigations with fruit crops have shown the great effectiveness of various growth regulators. According to Rakitin [2], spraying apples, pears, peaches, and apricots with 0.025-0.5% solutions of potassium α-naphthyl acetate hastened fruit ripening by 7 to 10 days. And at the same time it increased the yield by decreasing pre-harvest fruit drop by 20-30%. The utilization of these chemicals can also be very important in the culture of grapes. Aerial nutrition of grapes with many macroelements [7, 8], as well as microelements [9] indicates the great possibility of influencing the quantity as well as the quality of the yield by applying a whole series of chemical substances this way. This has been demonstrated by some investigations [10], including some by us [11-14].

With the object of first checking the effect of certain growth regulators on grapes, we set up some suitable experiments on the experimental farm "Chervonii khutor" in 1954. Grape variety Aligote was used. Solutions of potassium β-naphthoxyacetate (later indicated as I) and 2,4-dichlorophenoxy-α-butyric acid (later indicated as II) were applied aerially.

The grapes were first sprayed 2-3 days before mass flowering (June 18) and again when the berries had become the size of peas (July 12). Water solutions of each chemical were used; the estimated application per vine was 10^{-6} g of compound I and 10^{-7} g of compound II. About 200 ml of solution were used to spray each vine. No less than 50 grape vines were used in each variant, and there were four replicates. The control vines were sprayed with an equal volume of water.

The berries were analyzed twice, on September 4th, and again one day before harvest, on September 17th, according to the method we described earlier [11, 12]. A suitable time interval between analyses permits one to evaluate the dynamics of ripening to a certain degree. Pertinent data concerning the specific weight and volume of berries and their saccharinity and acidity are given in Table 1.

The data in Table 1 clearly show the appreciable hastening of ripening caused by compounds I and II. On September 4th the weight of the berries after treatment with compound I was 9% greater than the weight of the berries from the control vines, and after treatment with compound II it was 20% greater. The volume of berries increased correspondingly.

The growth regulators also had a specific effect on the saccharinity of the grapes; for example, with compound I there was an increase of 0.5% before harvest, and with compound II, 0.9% (absolute); at the same time there was a simultaneous decrease in acidity of the berries.

The acceleration of ripening is shown especially well in the figure. As we see from A and B in the figure and from Table 1, by harvest time (Sept. 17) the grapes on the control vines had just attained the characteristics

which had been noticeable two weeks earlier in grapes treated with compound I. The effect of compound II was even more noticeable. Hence, in the concentrations used, compound II was more effective than compound I. This is confirmed by data C and D in the figure and the data in the table concerning the measurement of

TABLE 1

The Effect of Potassium β-Naphthoxyacetate (I) and 2,4-Dichlorophenoxy-α-butyric Acid (II) on Grapes

Experimental design	Wt. of berries cm^3		Volume of berries cm^3		Saccharinity %		Titrated acidity in mg-eqv/l	
	9/4	9/17	9/4	9/17	9/4	9/17	9/4	9/17
Control	1.22	1.35	1.14	1.25	17.7	19.7	9.8	7.8
Compound I	1.33	1.50	1.24	1.38	18.3	20.2	7.8	6.3
Compound II	1.47	1.60	1.36	1.47	19.3	20.6	8.9	7.0

saccharinity and titratable acidity. The marked advantage of compound II in the concentration used is also evident. In that case, on September 4th the saccharinity was 1.6% greater than the saccharinity of the grapes from the control vines, and on September 17th it was 0.9% (absolute) greater. Note that the decrease in acidity after treatment with compound II was not as sharp as that after treatment with compound I. The acidity of the grapes from the control vines on the day of harvest was considerably greater than it had been even two weeks earlier in grapes on vines treated with compound I. All these data are evidence that ripening of grapes was accelerated after treatment with the compounds studied.

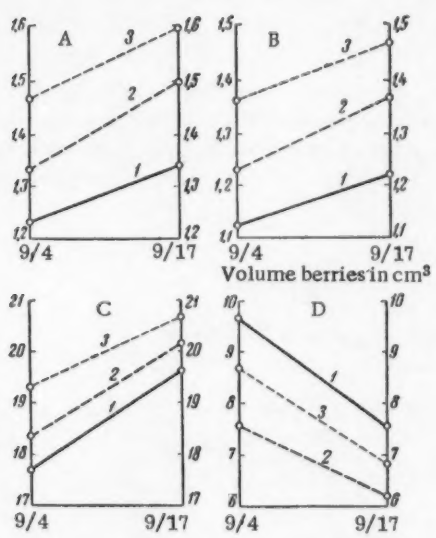

Fig. 1. The effect of some growth regulators on the growth of grape, variety Aligote, following aerial application.
1) Control; 2) potassium β-napthhoxyacetate in a concentration of $2 \cdot 10^{-6}$ g per vine; 3) 2,4-dichlorophenoxy-α-butyric acid in a concentration of $2 \cdot 10^{-6}$ g per vine.
A – Weight of berries in g; B – volume berries in cm^3; C – sugar content in %; D – titrated acidity in mg-eqv/l

Let us assume that one of the most important reasons for the favorable effect of growth regulators on the growth of grapes is, as we have also shown for the microelements [9], the effect of the specific chemical substance on the enzyme system of the plant. Under the influence of these substances the enzymes are no longer adsorbed but are dissolved in the dispersal media, and accelerate the hydrolytic processes in the plant organs.

In addition to such important factors as the type of plant, duration of treatment, climatic conditions, etc., the dosage or concentrations of the regulators was a primary factor at the time of the determinations. Although we do not by any means maintain that the concentrations of each compound that we used were optimal, we assume that a significant increase in the dosage would produce deleterious effects. It is no doubt because of this that many unsatisfactory results have been observed when these chemicals have been used previously.

We tried using compounds I and II in concentrations 100 to 1000 times greater on a limited number of grape plants of the same variety; the vines were either sprayed, or else these compounds were added to the soil. Without dwelling in detail on the data, we will just mention that some of the suitable concentrations had a favorable

effect on the fruiting of grapes and significantly increased the number of berries on the vines. However, the berries were small and contained considerably less sugar; the saccharinity of these grapes before harvest was 1.6-1.7% (absolute) lower than the saccharinity of grapes from the control vines. In our opinion, this can be explained by the retardation of ripening.

TABLE 2

The Aftereffect of Potassium β-Naphthoxyacetate (I) and 2,4-Dichlorophenoxy-α-butyric Acid (II) on Grapes

Experimental design	Weight of berries in g	Volume of berries in cm^3	Saccharinity in %	Titratable acidity in mg-eqv/l
Control	1.36	1.29	18.45	9.85
Compound I	1.43	1.33	19.45	9.05
Compound II	1.45	1.35	19.05	9.05

We also studied whether the same aerial application of solutions I and II (in concentrations indicated in the beginning of the paper) had an effect on the progress of fermentation of the grape wort. As we know, some chemical compounds, as well as microelements as we have shown, can either retard or accelerate fermentation. In our experiments compounds I and II (no noticeable variation between them was observed) which had entered the grapes retarded fermentation for one-two days. For example, if the period of vigorous fermentation in the grape wort from the control plants at certain temperature conditions ended the ninth day, it ended on the tenth or eleventh day in the wort from the treated vines.

As we noted above, all the experiments were done in 1954. The chemical compounds which entered the plants had an effect on the initiation of flower buds as well as other factors of development which could affect the yield the following year. Therefore, in 1955 the aftereffect of compounds I and II on the same specimens was studied. The results of the analyses performed on September 27th before the harvest are given in Table 2.

TABLE 3

Glucoacidimetric Indicators in Grape as the Direct Effect and as an Aftereffect of Potassium β-Naphthoxyacetate (I) and 2,4-Dichlorophenoxy-α-butyric Acid (II)

Experimental design	Sept. 4, 1954	Sept. 17, 1954	Sept. 27, 1955
Control	1.80	2.52	1.87
Compound I	2.34	3.20	2.14
Compound II	2.17	2.94	2.10

As we can see from the data in Table 2, the growth regulators did produce some aftereffects on the growth of grapes. It is true that compounds I and II increased the weight of the berries to a considerably smaller degree (5-6%) than they did the year of their immediate effect (see Table 1). However, the increase in saccharinity with both substances which corresponded to 1% and 0.6% (absolute), with a simultaneous decrease in acidity, was quite noticeable.

Hence, both growth regulators also somewhat accelerated the ripening of grapes as an aftereffect. This is especially clear from the results obtained after calculating the glucoacidimetric indicator which represents the ratio between the percentage of saccharinity and total acidity in mg-eqv/l (Table 3).

A comparison of the glucoacidimetric indicators in 1954 (especially just before harvest) and those in 1955, clearly reveals the greater prevalence of saccharinity over acidity when either of the regulators were used, as compared with the indicators in grapes on the control plants. The same predominance was greater before harvest time in 1954; in 1955 compounds I and II also produced an aftereffect, but it was considerably lower. It should also be taken into account that the climatic conditions in 1955 were not very favorable for the growth of grapes and the ripening period was considerably prolonged.

It is very important to note that aerial applications of various solutions of growth regulators do not require great expenditures, and are quite practicable under cultural conditions. Taking all the other factors into consideration, a suitable effective reaction can express itself as an increase in the yield of grapes and an improvement of their quality.

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THE EFFECT OF THE VARIETY OF THE POLLINATORS AND OF POLLEN MIXTURES ON VARIOUS PHYSIOLOGICAL PROCESSES IN APPLE SEEDLINGS

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At the present time many investigators are studying the effect of different varieties of pollinators and mixtures of pollen on the viability of the progeny in fruit and berry crops, especially in apple. This important problem is the practical significance in the growth of valuable uncultivated material in the nurseries, and also in the selection of parent plants in selective breeding. Furthermore, in practically all the previous investigations, vitality was judged only by the growth and overall conditions of the seedlings, and little attention was paid to the physiological processes occurring in the seedlings in relation to the mode of pollination. We therefore set up a special experiment concerned with the study of the effect of the variety of the pollinators and that of pollen mixtures on various physiological processes occurring in apple seedlings.

The work was done during 1953-1955 on the experimental production farm at the I. V. Michurin Scientific Experiment Institute of Horticulture. The effects of the variety of the pollinators and pollen mixtures on the water relations and photosynthetic activity in apple were studied.

METHODS

The half-leaf method (Sachs method) was used to measure photosynthesis in the leaves of the seedlings. The water content of the leaves was measured at the same time. The capacity of the leaves to absorb water when they are immersed in water was measured according to Litvinov's method [1]. Ten leaves without petioles were picked from the central part of typically growing seedlings for each variant and carried quickly to the laboratory in lightly moistened fabric. After they were weighed in the laboratory, the leaves were immersed in water. After four hours the leaves were removed, the drops of water were absorbed with filter paper, and the leaves were weighed again. The capacity of the leaves to absorb water was judged by the differences in weight. The experiment was performed in duplicate. One-year-old hybrid apple seedlings from various combinations of crossbreeding were used as experimental material.

The Effect of the Variety of Pollinators and Pollen Mixtures on the Water Relations of Apple Seedlings

The normal progress of many physiological processes depends on the degree of hydration of the leaf. Gunalo [2] considered that a measurement of the hydration of the leaves could be recommended as one of the indicators which characterize the physiological condition of the plant. Smetannikova [3], working with lucerne, showed that when air temperature increased during the day, the water content of the leaves decreased in various species of lucerne, and vice versa.

The degree of water saturation of the leaves has an effect on the progress of enzymatic activity [4]. The water relations of the leaves determine, to a certain degree, the resistance of the plant to unfavorable soil and climatic conditions. According to Eremeev's data [5], drought-resistant fruit-bearing plants are characterized by an increased capacity for retaining water in the tissues during wilting. This indicates the importance of being familiar with the water relations when studying the vitality of one or another plant organism.

During 1954-1955 we studied the water content of the leaves of one-year-old hybrid apple seedlings at various times of the day in relation to the variety of the pollinators. As the experiment showed, the leaves of the hybrid seedling were characterized by the lowest water content and the greatest water deficiency during the daylight hours. In the meantime the leaves of seedlings from seeds resulting from self-pollination contained 1-4% less water than the leaves of seedling resulting from cross-pollination. Leaves of seedlings resulting from self-pollination had the greatest water deficiency during the day. Leaves of seedlings from seeds resulting from pollination with mixtures of pollen were hydrated the best of all. For example, the leaves of seedlings obtained from seeds resulting from self-pollination of Korichnaia kitaika X Korichnaia kitaika contained 61.3% water on June 29th at 9 o'clock, the leaves of seedlings obtained from seeds resulting from cross-pollination (Korichnaia kitaika X Grushovka moskovskaia, Korichnaia kitaika X Rekord Michurnina) contained more water (63% and 62.5%). Leaves of seedlings obtained as the result of pollinating with a mixture of pollen (Korichnaia kitaika X Grushovka moskovskaia + Papirovka + Rekord Michurina) contained the greatest amount of water (164.8%). By 1 : 00 P. M. the leaves of all the variants had lost some water because of the rapid rate of transpiration by this time; however, even at that time the rule remained as before. Leaves of seedlings from seeds resulting from self-pollination contained 58.5% water, leaves of seedlings from seeds resulting from cross-pollination contained 59.2% and 59.7% water, the leaves of seedlings from seeds resulting from pollination with mixture of pollen were the best hydrated (62.5%).

Analyses of these variants on August 15, 1954 revealed that the picture of the water content of the leaves remained the same as it had been on June 29th. Analagous results concerning the water content of the leaves were obtained with seedlings of Pamiat Michurina and Bel'fler-kitaika.

In 1955 we measured the water content of the leaves from seedlings of the variety Skryzhapel krupnyi. And in this case also the leaves of seedlings resulting from self-pollination contained less water, especially during the day, than the leaves of seedlings resulting from cross-pollination. When the water-absorbing capacity of the leaves was measured following their immersion in water, the same rule also applied again. The leaves of seedlings resulting from self-pollination had a greater absorbing capacity than the leaves of seedlings resulting from cross-pollination, even in the morning and even when the water deficiency was comparatively negligible. This absorbing capacity was the lowest in the leaves of seedlings obtained from seeds resulting from pollination with a mixture of pollen. The variation in absorbing capacity among the variants appeared even more clearly during the day. For example on June 30th the leaves of the seedling Korichnaia kitaika in the self-pollinating variant absorbed 159 mg water per g of leaf during the daylight hours after the leaves had been in water 4 hours; there was considerably less absorbed by those resulting from cross-pollination; 146 mg in the variant pollinated with Grushovka moscovskaia and 149 g in the variant pollinated with Rekord Michurina. Even less was absorbed by the variant resulting from pollination with a mixture of pollen – 128 mg.

An analysis of the figures concerning the degree of water absorption by the leaves on August 7th revealed that the absorbing capacity of the leaves changed during the course of the day. The absorbing capacity was the lowest in the morning: in Korichnaia kitaika seedlings it was from 71 to 85 mg, and in Pamiat Michurina seedlings it was from 83 to 109 mg water; during the day (at 1 : 00 P. M. when transpiration increased and the leaves became hydrated, their absorbing capacity increased to a maximum. At that time the water absorbing capacity in the leaves of Korichnaia kitaika seedlings varied from 97 to 127 mg in the different pollination variants, in the leaves of Pamiat Michurina seedlings it was from 116 to 161 mg, and finally in the evening (in this case 6 : 00 P. M.) transpiration became reduced, and the leaves began to become hydrated; this was indicated by the decrease in absorbing capacity of the leaves (in Korichnaia kitaika seedlings it was from 83 to 106 mg and in Pamiat Michurina seedlings it was from 99 to 126 mg).

In 1955 we studied the water-absorbing capacity of leaves in relation to the pollinators in the seedlings of two varieties: Diana and Skryzhapel krupnyi. The greatest amount of water was absorbed by the leaves of seedlings obtained as the result of self-pollination of Diana. In the morning these leaves absorbed 85 mg water, during the day – 124 mg. The leaves of seedlings from the same variety of Diana, but which were obtained as the result of cross-pollination, absorbed less water in the morning (72 and 81 mg); during the day they absorbed 123 and 106 mg. Leaves of seedlings obtained as the result of pollinating with a mixture of pollen had a low water-absorbing capacity: in the morning they absorbed only 64 mg water and during the day – 108 mg per gram of leaf weight.

Similar results concerning the absorbing capacity of leaves were obtained with seedlings of Skryzhapel krupnyi.

The Effect of the Variety of the Pollinators and a Mixture of Pollens on the Photosynthetic Activity of Apple Seedlings

The productivity of photosynthesis is an important indicator of plant vitality; it changes to a considerable degree in relation to changing conditions. Ivanova [6] showed that the productivity of photosynthesis represents the product of three values: intensity, duration, and functioning leaf area.

The hydration of the assimilating apparatus influences the intensity of photosynthesis to a considerable degree. The photosynthetic productivity decreases if dehydration exceeds a certain limit [7, 8]. It has been shown that the higher the osmotic pressure of the leaf cells, the lower the rate of photosynthesis [9, 10].

Our problem was to show the effect of the variety of the pollinators and mixtures of pollen on the rate of photosynthesis in one-year-old apple seedlings. We used the increase in dry weight of the leaves as an indicator of the rate of photosynthesis.

Investigations revealed that the seedlings obtained as the result of pollination with mixtures of pollen had the highest rate of photosynthesis; those from seeds resulting from self-pollination had the lowest rate (see table).

The table shows that on June 29th, seedlings of Korichnaia kitaika resulting from self-pollination accumulated 0.85 g dry material per m^2 of leaf area per hour from 10 A. M. to 2:00 P. M., while at the same time seedlings resulting from cross-pollination accumulated considerably more dry material during the same time (1.20-1.30 g). The highest rate of photosynthesis occurred in seedlings obtained from seeds resulting from pollination with a mixture of pollen from Grushovka moskovskaia + Rekord Michurina (1.42 g). This regularity was also observed on August 15th, 1954 in this variety as well as in other varieties.

TABLE

The Accumulation of Dry Material by the Leaves of Hybrid Apple Seedlings in Relation to the Variety of the Pollinators

(in g per 1 m^2 leaf area per hour)

1954 Experiment

Variety of pollinators	June 29th, 10 A.M. to 2:00 P.M.	August 15th, 9 A.M. to 1:00 P.M.
Pollinated variety – Korichnaia kitaika		
Korichnaia kitaika	0.85	1.11
Grushovka moskovskaia	1.20	1.35
Rekord Michurina	1.30	1.18
Grushovka moskovskaia + Rekord Michurina	1.42	1.40
Free pollination	—	2.60
Pollinated variety – Pamiat Michurina		
Pamiat Michurina	0.32	0.64
Pomon-kitaika	0.48	—
Autonovka obyknovennaia	1.12	1.03
Autonovka obyknovennaia + Pepin shafrannyi + Severnyl sinap	—	1.70
Free pollination	0.79	1.22
Pollinated variety – Bel'fler-kitaika		
Sentiabr'skoe polosatoe	0.86	—
Koreianka	0.60	—
Osenniaia radost + Sentiabr'skoe polsatoe + Koreianka	1.09	—
Free pollination	0.94	—

The analyses in 1955 on seedlings of variety Diana and Skryzhapel strongly supported the previous data. Seedlings of Diana resulting from self-pollination had the lowest rate of dry material accumulation in the leaves (1.00 g). Seedlings obtained from seeds resulting from cross-pollination had a higher rate of dry-material accumulation (1.11 and 1.41 g), and seedlings obtained from seeds resulting from pollination with mixtures of pollen had the highest rate of dry-material accumulation in the leaves (1.80 g). Data obtained with seedlings of the variety Skryzhapel were even more indicative (from self-pollination – 0.4 g, from pollination with pollen mixtures – 1.42 g per m^2/hr).

The higher degree of hydration in apple seedlings obtained from seeds resulting from pollination with mixtures of pollen, and also the increased rate of photosynthesis in these seedlings indicates their high vitality. Proceeding from this we should also note that it is necessary to have 2-3 varieties of pollinators in the maternal seed plantings of apple together with pairs of the main variety suitable for that place; this guarantees pollination with a mixture of pollens under natural conditions in each instance, and significantly increases not only the percentage of fruits set, but also guarantees getting vital pairs of seedlings. It is also necessary to expand the use of mixtures of pollen in selective breeding since more vital hybrids are produced as the result.

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DEPENDENCE OF SOME ASPECTS OF NITROGEN METABOLISM IN PLANTS ON CONDITIONS OF PHOSPHORUS NUTRITION

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When studying the effect of the conditions of phosphorus nitrition on the metabolism of plants it is frequently necessary to exclude phosphorus from the nutrient medium for some time and then later to again place the plants in solutions containing phosphorus. Under such conditions when the plants previously deficient in phosphorus are transferred to the nutrient medium which contains all the necessary elements, including phosphorus, the leaves frequently exhibit characteristics of toxicity which are accompanied by the entrance of large quantities of phosphorus into the plants: the dissociation of pigments, the loss of turgor, and the death of certain parts of the leaves [1-6].

In this investigation we studied the causes of leaf toxicity produced by the preliminary phosphorus deficiency and the later addition of phosphorus. It was assumed that one of the causes might be the disruption of nitrogen metabolism in the plant as the result of changing the conditions of phosphorus nutrition. The experiments were done with sand cultures of corn, variety Voronezhskaia 76, using Hellriegel's nutrient solution. The plants were grown on nutrient solutions with either ammonium or nitrate sources of nitrogen and with different concentrations of phosphorus: 0P, 1P, and 3P.

Twenty-eight days after germination, when the plants growing without phosphorus had already begun to show signs of phosphorus deficiency, phosphorus was added. Observations on the growth of the plants revealed all the characteristic signs of toxicity four days after phosphorus was added to the the ammonium-containing solutions and five to six days after it was added to the nitrate solutions. An investigation on the forms of nitrogen present in the plants disclosed the fact that under conditions of phosphorus nutrition when ammonium nitrogen was available, the plants contained many amides and other non-proteinaceous nitrogen compounds, however the amount of ammonium present was low and usually amounted to ~ 0.1 mg per gram fresh weight. The ratio between protein nitrogen and non-protein nitrogen in these plants was strongly inclined toward the non-protein nitrogen. Hence, the synthesis of amides and amino acids in the plants during phosphorus deficiency was quite intensive, but the synthesis of newly formed proteins ceased. After the addition of phosphorus the plants recovered their capacity to synthesize proteins in two to three days, and the total content of protein nitrogen per plant three hours after the addition of phosphorus increased almost one and one-half times, on the average. The amount of amides and other non-protein nitrogen compounds decreased noticeably during this time, however, at the same time, a considerably amount of nitrogen in the form of ammonium entered the plants. During the further growth of the plants the amount of proteins, amides, and nonprotein nitrogen increased, but the ammonium which continued to enter the plant could no longer be fully utilized in organic synthesis and accumulated in the plant tissues. The ammonium nitrogen content three days after the addition of phosphorus was 0.211 mg, and after six days it was 0.406 mg per gram of fresh weight of the leaves. The accumulation of large quantities of ammonium resulted in the ammonium poisoning of the cells and was, we believe, the cause of the toxicity observed in the various plant tissues.

When nitrates were utilized during phosphorus deficiency, the reduction of nitrogen ceased almost entirely

and the processes of ammonium, amide, amino acid, and protein synthesis were limited by the rate of nitrate reduction. After the addition of phosphorus the entrance and reduction of nitrate increased, and in certain parts of the leaves there may have been conditions when the rate of ammonium formation exceeded the possibile rate of its further conversion; this might have resulted in the accumulation of excessive amounts of ammonium nitrogen in certain cells and tissues and consequently poisoned the plants. In our experiments with nitrate-containing nutrient solutions the amount of ammonium nitrogen in the entire aerial part of the plant increased four-fold 6 days after phosphorus was added (from 0.623 to 0.149 mg per g fresh weight); this, evidently, was inadequate to induce partial poisoning of the leaf tissues and retard the growth of the plant.

Observations on the growth of plants which were deficient in nitrogen as well as phosphorus simultaneously revealed that after nitrogen and phosphorus were added, the plants very quickly regained their normal dark green color, grew rapidly, and exhibited no signs of toxicity. The content of protein as well as nonprotein forms of nitrogen increased rapidly in these plants, however there was no observable accumulation of ammonium.

The data obtained lead us to conclude that toxicity was accompanied by the accumulation of excessive amounts of ammonium nitrogen in the plants, and the probable cause of poisoning when phosphorus was added after the initial phosphorus deficiency was the disruption in nitrogen metabolism, especially ammonium poisoning of some of the plant tissues.

When a high dose of phosphorus (3 parts according to Hellriegel) was added during planting, the characteristic toxicity was observed in the leaves during the first 15-20 days after the seeds germinated. There was considerably more ammonium in the leaves of these plants than in those with a normal phosphorus supply.

While we were making observations on the appearance of toxicity, we noticed that it occured first in the old leaves, and also in the older parts of the leaves. Determinations of ammonium nitrogen content revealed that those parts of the leaves which exhibited poisoning first, and to the greatest degree, also contained a great amount of ammonium. Usually the content of ammonium in the tips of the corn leaves was two to three times that of tissues at the base of the leaves. Signs of poisoning began to appear when the ammonium content in various cells of the leaves exceeded some value which was not yet harmful to the plant. According to our data with corn, this limit was an ammonium nitrogen content of 0.2 mg per gram fresh weight of the leaf during nitrate utilization, and about 0.3-0.4 mg during ammonium utilization.

Corn leaves were subjected to poisoning due to ammonium toxicity when comparatively large amounts of ammonium accumulated in the plants. It was of interest to check whether there was poisoning due to ammonium, and whether there were any signs of toxicity in those plants which have an acid plant sap, accumulate a large quantity of organic acids in their tissues, and according to Kul'chera's data (cited by Prianishnikov [7]) posses the capacity to reduce ammonium and combine it in the form of ammonium salts of organic acids. We used sorrel as an example of such a plant.

The design of the experiment with sorrel was similar to that used with corn. Phosphorus was added 35 days after the plants were transferred to a nutrient solution. The signs of toxicity began to appear in the sorrel leaves four days after phosphorus was added when ammonium was the nitrogen source, and in six or seven days when nitrate was the nitrogen source. The determinations of the forms of nitrogen present in sorrel disclosed the same rules which were observed in the experiments with corn; toxicity was accompanied by an increase in ammonium nitrogen content; this resulted in ammonium poisoning of the plants. It should be noted that the total content of ammonium in sorrel leaves was considerably higher than in corn and usually consisted of 0.8-0.9 mg per g fresh weight of leaf tissue when ammonium was the nitrogen source; this would be absolutely lethal for corn. When toxicity was apparent, the ammonium content of the sorrel leaves had increased sharply. Seven days after phosphorus was added when ammonium was the nitrogen source it consisted of 2.65 mg per g fresh weight; this is equivalent to a 0.2N solution of an ammonium salt in the plant tissues. Such a high ammonium content does not kill plants like sorrel.

We performed an experiment with corn in liquid culture in order to study the relationship between the causes of toxicity during nitrogen metabolism in greater detail. The experiment was set up with two variants: $P_{1/10}$, 1N and $P_{1/10}$, $N_{1/10}$. Sulfate of ammonia was the nitrogen source; gypsum and lime were also added. The nutrient solutions were changed after 34 days. In part of the vessels of each variant the nutrient solution was exchanged for one containing 3P and 1N, in the other part of the vessels only phosphorus was added without nitrogen. The samples were taken and analyzed the day the solutions were changed, and again three and seven

days after they were changed. The data obtained are given in Table 1.

TABLE 1

Nitrogen Forms Present in Corn Leaves (in mg N per g fresh weight)

Number of variant	Conditions of nutrition: before Aug. 27 (1-34 days)	Conditions of nutrition: after Aug. 27 (after 35th day)	Date samples taken for analysis	Ammonium	Amide	Nonprotein	Protein	Total
I	$P_{1/10}$ 1N	3P 1N	Aug. 27	0.136	0.431	1.27	3.13	4,40
			Aug. 30	0.282	0.373	1.19	3.25	4,44
			Sept. 3	0.472	0.643	1.93	4.63	6,56
II	$P_{1/10}$ 1N	3P	Aug. 27	0.136	0.431	1.27	3,13	4,40
			Aug. 30	0.097	0.216	0.75	2,90	3.65
			Sept. 3	0.00	0.127	0.51	2,84	3,35
III	$P_{1/10}$ $N_{1/10}$	3P 1N	Aug. 27	0.024	0.092	0.29	1,80	2,09
			Aug. 30	0.051	0.103	0.77	1.85	2,62
			Sept. 3	0,023	0.264	1.19	3,22	4,41
IV	$P_{1/10}$ $N_{1/10}$	3P	Aug. 27	0.024	0 092	0.29	1,80	2,09
			Aug. 30	0.032	0.040	0,32	1,60	1,92
			Sept. 3	0.00	0.058	0.32	1.24	1,56

Note: Analyses on Aug. 27 were made before changing the media.

The results show that when the scheme of nutrition was $P_{1/10}$, 1N, 3P, 1N the amount of all forms of nitrogen increased rapidly in the leaves. The increase of ammonium nitrogen content was especially sharp and was accompanied by an intense toxicity in the leaves. No signs of toxicity were observed in any of the other variants and there was no accumulation of ammonium. Hence, toxicity was observed only when the plants were supplied with an adequate amount of nitrogen during the period of phosphorus deficiency and after phosphorus was added to the nutrient solution.

TABLE 2

The Rate of P^{32} Movement Into Corn Plants (in thousands of counts/min/100 mg fresh weight)

No. of variant	Conditions of nutrition	Time in days after addition of P^{32}: 1	2	3
I	0P 1N am. + 3 P^{32}	3.3	10.3	21.9
II	0P 1N nitrate + 3 P^{32}	4.1	9.2	13.0
III	0P 0N + 3 P^{32} 1N am.	1.5	4.2	8.5
IV	0P 0N + 3 P^{32} 1N nitrate	0.9	2.0	4.4

In our experiments we showed that toxicity occurred not only when there was an accumulation of ammonium in the plant tissues, but also when a large amount of phosphorus entered the plant.

Determinations of the rate of phosphorus movement into the plants using P^{32} (the data from one of these experiments are given in Table 2) disclosed that the rate of phosphorus movement into plants of variants I and II, which had no nitrogen available during phosphorus deficiency, was several times greater than it was in the plants which were simultaneously deficient in phosphorus and nitrogen and then had phosphorus and nitrogen added (variants III and IV). From these data it follows that during nitrate utilization the amount of phosphorus entering the plant was lower than it was during the utilization of ammonium; this was associated with the appearance of less toxicity when nitrates served as the nitrogen source.

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METHODS

AN APPARATUS FOR THE GASOMETRIC DETERMINATION OF PHOTOSYNTHESIS IN FIELD CONDITIONS

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Although gasometric methods of measuring photosynthesis are the most suitable, they are still not well enough developed for field work. The difficulties grow with the increase in number of the plants being studied simultaneously. We have designed and constructed an apparatus and worked out a method of measuring photosynthesis in field conditions.

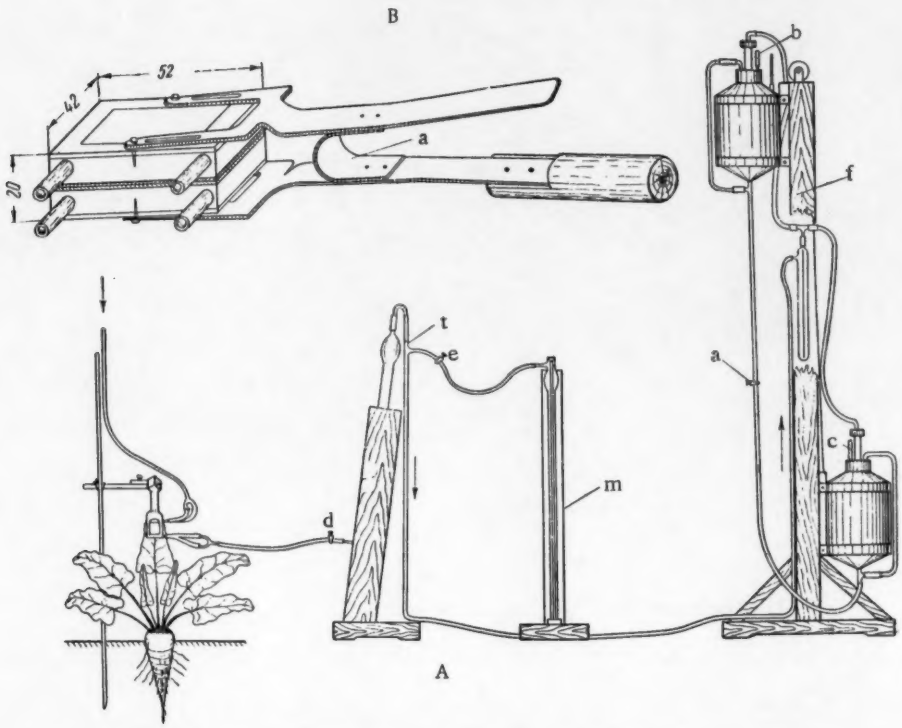

Fig. 1. Diagram of the apparatus for measuring photosynthesis in field conditions.
A) General view; B) clip-on chamber for 10 cm^2 (explanation in text).

In the course of five years' work the apparatus has been tested in numerous studies of photosynthesis in various crop plants.

Below we give a description of its principal components: chambers, absorbers for carbon dioxide and aspirators (see Fig. 1).

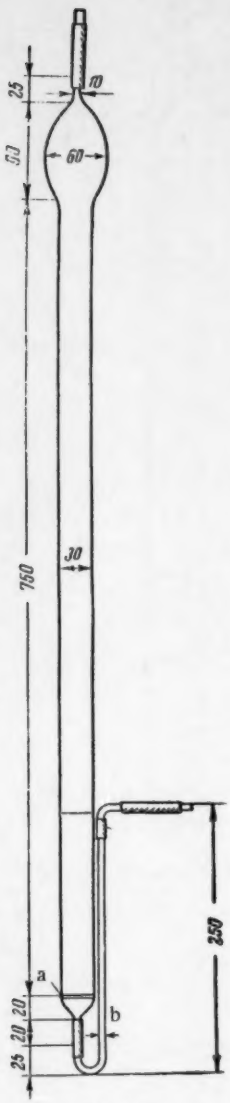

Fig. 2.

Chambers. In field conditions the most convenient are clip-on chambers [1, 2] (we used ones which covered 5 cm^2 and 10 cm^2 of the leaf surface). They are made from clear plastic and are composed of two identical frames held tightly together by means of a spring. The edges of the frame in contact with the leaf have strips of sponge rubber glued on to them. In one wall of each frame their are two openings for the entry and exit of air. Each of these opens into the chamber through three small orifices 2 mm in diameter. Aluminum tubes (d = = 4 mm) are screwed into the holes in the chambers and are connected to each other in pairs by a glass T-piece and rubber tubing. In this way the upper compartment of the chamber is united with the lower.

Adsorbers. We used Rikhter absorbers [3], the construction of which was altered to make them suitable for field conditions (Fig. 2). As a distributor in the absorber we used a glass filter No. 1 (d = 25 mm) of the 'Druzhnaia Gorka' factory (Fig. 2,a). The bent glass tube connected to the lower part of the absorber is bound to it with thread above a rubber ring (Fig. 2,k). The assembled absorbers are mounted in wooden stands, four in each, inclined at an angle of 82°, which assists the circulation of the solution (Fig. 1).

For obtaining more accurate results in the carbon dioxide determination we chose for the absorbers porous plates which had the same resistance to air flow. With a flow of 1 liter/min the differences in resistance must not exceed 5 mm of water.

In making the absorbers it is essential to use glass resistant to alkalis. In this respect glass No. 23 is perfectly suitable and 'molybdenum' glass quite useless.

New absorbers must be tested by measuring the carbon dioxide in outside air and comparing the results obtained with parallel determinations by as many absorbers as possible.

Aspirators. Six-liter aspirators for drawing the air through and at the same time measuring its volume are united in fours on one stand. They are made of galvanized iron, painted white and numbered (Fig. 3). The wooden stands each have two T-shaped grooves in which slide similarly shaped metal strips fastened to the aspirators and joined in pairs by a cord over a pulley. The pulleys are mounted above each rail. The length of the cord is chosen so that when one aspirator is up the other is below at a distance of 10-15 cm from the lower end of the rail.

The assembled aspirators are filled with water and calibrated to an accuracy of 0.1 liter, by painting marks on the water gauge. The bottom connections of the aspirators are joined together in pairs by rubber tubing, one aspirator being in the up and the other in the down position. For checking the rate of air flow through the chamber and absorber each pair of aspirators is connected to a rheometer fixed on the inside of the frame of the stand. The portable forms of rheometer designed by us are depicted on Fig. 4. The prepared rheometers are calibrated in this way: distilled water is poured through the top of the rheometer to give a column 30-35 mm high, the water level is noted (zero point) the outer tube c is connected to the outlet of a gas meter, the inner d to an aspirator or pump, and by varying the speed we obtain 4-5 values for different heights risen by the water. From the obtained results we plot a curve which is then used for constructing the scale. The scale, accurate to within 0.01 liter/min, is marked on a paper which is glued to the outer tube of the rheometer.

The aspirators are tested for air-tightness by means of a water manometer (Fig. 1,m); it is used for measuring the underpressure in the aspirators during the measurement of photosynthesis and is mounted on a wooden stand with a scale made of millimeter graph paper; it is filled with water up to the bulb and the level is set against the zero scale reading.

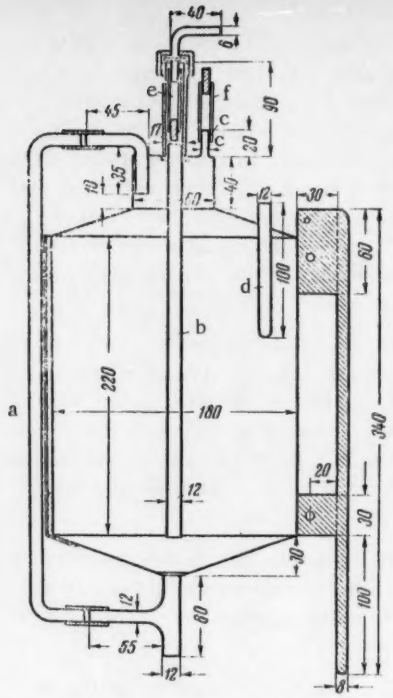

Fig. 3. Aspirator.
a) Water gage; b) Mariotte tube; c) connecting tube; d) brass tube for thermometer; e) Bunsen valve and screw cap with Γ-shaped tube; f) Bunsen valve for outlet of air (figures – sizes in mm).

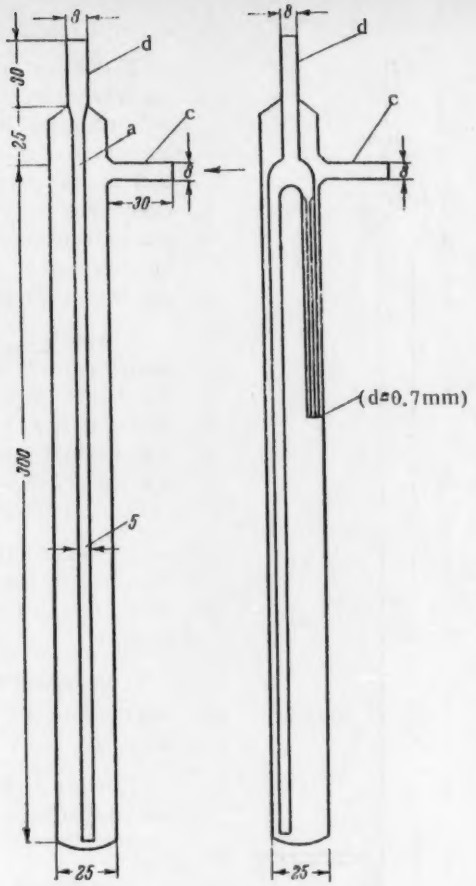

Fig. 4. Rheometers (explanation in text).

Carbon Dioxide Determination

For determining the carbon dioxide in the air we used the principle based on the direct titration of a baryta solution with atmospheric carbon dioxide in the presence of phenolphthalein [1]. But even in the new improved form [2] it cannot be used for the measurement of photosynthesis owing to the large errors arising from the incomplete absorption of carbon dioxide even with low rates of air flow. In using this method with the above absorbers to determine the carbon dioxide in 20 liters of air we obtained the results given in Table 1.

We see from Table 1 that the titration with atmospheric carbon dioxide in the presence of phenolphthalein does not give accurate results. The error arises from the incomplete absorption of the carbon dioxide at the end of the experiment when the concentration of alkali is greatly reduced. The size of the error depends on the concentration of carbon dioxide in the air, the baryta concentration and the rate of air flow through the absorber. For reducing this error we used thymolphthalein, the transitional pH of which is one-and-a-half units higher than that of phenolphthalein (9.4 to 10.6). In the complete decolorization of thymolphthalein (pH −9.4) the concentration of hydroxyl ions in the solution is 25 times higher than in the decolorization of phenolphthalein (pH −8) and is equal to $2.5 \cdot 10^{-5}$, which still ensures the complete absorption of carbon dioxide. Determinations of carbon dioxide in the air by titration of baryta in the presence thymolphthalein and the volumetric method gave the results shown on Table 2.

We see from Table 2 that the method is no less accurate than the volumetric method (+0.3%) and may be used both for the determination of carbon dioxide in the air and for the measurement of photosynthesis.

In order to eliminate the abundant foam which hinders the increase of the air flow through the absorbers, and to avoid the deposition of indicator sediment at the end of the reaction, we added 1.5-2% of ethyl alcohol to

TABLE 1

No. of expt.	CO_2 found in %		Difference	Error in%
	on titration with atmospheric carbon dioxide	control		
1	0.0236	0.0298	0.0062	−20.8
2	0.0222	0.0296	0.0074	−25.0

TABLE 2

No. of expt.	CO_2 found in mg per 20 liters of air		Difference	Error in%
	on titration with atmospheric carbon dioxide	volumetric method		
1	12.42	12.36	+0.06	0.49
2	12.24	12.30	−0.06	0.49
3	12.32	12.20	+0.12	0.98
4	12.20	12.10	+0.10	0.83
5	12.38	12.26	+0.12	0.98
6	12.26	12.22	+0.04	0.33
7	12.28	12.24	+0.04	0.33
8	12.22	12.18	+0.04	0.33
9	12.16	12.22	+0.06	0.49
10	12.12	12.14	−0.02	0.16
Mean	12.26	12.22	+0.04	+0.33

the solution. In these conditions it is possible to draw through the absorber up to 1 liter of air per minute without the formation of large bubbles.

With this method the error of the mean is

$$m = \sqrt{\frac{0.0792}{10.9}} = \pm 0.03 \text{ mg.}$$

But since the rate of photosynthesis is determined by the difference between the carbon dioxide content of the air before and after the chamber, then the mean error of this difference will be:

$$md = \sqrt{m_1^2 + m_2^2} = \pm 1.41m = \pm 0.042 \text{ mg,}$$

which in comparing the results of measurement of the photosynthesis in two plants gives an error of 0.06 mg and a maximal error of ±0.18 mg CO_2 or 0.3 liter of air. Therefore, in using this method, we can consider as significant only differences in carbon dioxide assimilation of the two plants, which within the time of the experiment have values exceeding 0.18 mg CO_2. Hence we see that it is possible to detect a 10% difference in photosynthesis by an experiment in which the assimilation of carbon dioxide would not be less than 1.8 mg (3 liters of air), i.e., 30 to 60 minutes for a 10 cm^2 chamber.

Preparation of Apparatus and Measurement of Photosynthesis

100 ml of 0.008 N $Ba(OH)_2$ solution containing thymolphthalein and butyl alcohol is poured into clean dry absorbers filled with air freed of carbon dioxide. In the apparatus for filling the absorbers we use a pipette with an automatic setting zero point. When the pipette is filled we squeeze with the left hand the rubber tubing at the top of the absorber and remove the glass rod. We insert the end of the pipette and add the solution to the mark, at the same time allowing air to escape through the rubber tubing. Taking the pipette out of the absorber we squeeze the rubber tubing and close it with the glass rod. The filled absorbers on the stand are conveyed to the place of experiment. There one of each pair of aspirators is filled with water through the Mariotte tube by means of a siphon. Unscrewing the screw cap we add water until it begins to flow from tube b (Fig. 3). The screw cap is tightened, the clip is closed, and the water-filled aspirator is drawn up to the top, where it is fixed by means of a pin inserted in a hole in the stand. The aspirators are connected by rubber tubing to the upper ends of the absorbers, and the chambers to the lower tubes which are closed beforehand by screw clips d (Fig. 1,A). The assembly is then ready for operation.

The measurement of carbon dioxide assimilation is carried out in this way: clip a is opened a little, clips d are removed and the air starts to flow with a speed of 0.6 or 0.8 liter/min. As soon as the aspirators settle to the same speed of air flow, the chambers are clipped to the leaves under investigation and the time noted on a stop watch. The aspirator is quickly moved down when the water is flowed out of it, the clip a first being closed. The valves ensure a continuous flow of air (one operator can easily handle four pairs of aspirators). During the experiments the number of changes of the aspirators is recorded, as well as the air temperature in them after each emptying. The underpressure is measured twice, once during the course of the experiment and once at the end. For this purpose there is inserted between the aspirators and the absorbers a T-tube t to which a water manometer m is connected. The stem of the T-tube is closed with a clip e, which can be opened to measure the underpressure. The experiment is continued until the solution in one of the absorbers begins to pale appreciably. The chambers are then removed from the leaves and the time noted. The titration of solution in the absorbers

is completed with atmospheric carbon dioxide, which is drawn up till the solution is decolorized, the rate of flow towards the end being reduced in such a way that the lower part of the absorber contains a layer of colored solution free of bubbles. In completing the titration it is possible to use a standard giving the color of the indicator at pH 9.4 (for 100 ml of distilled water we take 1 ml 1% solution of $CuSO_4 \cdot 5H_2O$; 0.1 ml of 0.1 N solution of $K_2Cr_2O_7$ and 1 ml concentrated ammonia solution: the solution obtained is poured into an absorber, well stoppered and set up beside the experimental absorbers). As soon as the solution becomes clear, clip a is closed, the underpressure in the aspirator is measured and the volume of water which has flowed is read from the water gauge. At the same time we determine by the same method the CO_2 content of the air, as well as the barometric pressure, temperature, humidity of the air and the solar radiation.

Calculations

The amount of air drawn through the absorber (vt) is calculated by multiplying the volume of the small aspirator to the pair by the number of shifts of the aspirators with the addition of the reading from the water gauge in the last aspirator. The volume obtained is brought to N. T. P. (0° and 760 mm) according to the following formula:

$$V_n = \frac{[P - (W + H)]\,Vt}{760\,(1 + 0.00366\,t_{cp})},$$

where P – atm. pressure

H – underpressure in aspirator in mm Hg;

W – tension of water vapor in aspirator in mm Hg;

Vt – volume of air in liters drawn through absorber;

t – temperature of air in aspirator (mean).

From the volumes of air, brought to N.T.P., drawn through 100 ml $Ba(OH)_2$ in the experiment (v_1) and the control (v_2) we calculate the amount of carbon dioxide assimilated in mg per hour for 100 cm of leaf surface from the formula:

$$CO_2 \text{ mg/hr} = \frac{132 \cdot 10^3 N \cdot A\,(v_1 - v_2)}{v_2 \cdot m \cdot C},$$

where N – normality of oxalic acid solution;

A – volume of oxalic acid in ml, used up in the titration of 100 ml of 0.008 N solution of $Ba(OH)_2$;

v_1 – volume of air in liters, brought to N.T.P., drawn through 100 ml of $Ba(OH)_2$ in the experiment with the plant;

v_2 – volume of air in liters drawn through 100 ml of $Ba(OH)_2$ in control;

m – duration of experiment in min;

C – investigated area of leaf in cm^2; $132 \cdot 10^3 = 22 \cdot 60 \cdot 100$ – product of normal titer of carbon dioxide in mg, 60 min and 100 cm.

This method measures only the 'observable' (productive) photosynthesis.

The absorbers may be used five to six times without washing. After the experiment all the solution is removed from them and they are then filled with fresh solution. So that air containing CO_2 does not enter them, the upper end of the absorber is connected to a Tishchenko flask containing a NaOH solution and a soda lime tower. The lower end of the absorber is connected to a Bunsen flask which is tightly stoppered, and when the air is sucked out of the flask all the solution is drawn out of the absorber. The absorber is then disconnected and it is ready for filling with $Ba(OH)_2$ and for use again.

Reagents

0.008 N solution of barium hydroxide is prepared by dissolving 13 g of $Ba(OH)_2 \cdot 8H_2O$ and 5 g of $BaCl_2 \cdot 2H_2O$ in 10 liters of boiled distilled water. To this is added 200 ml of 0.05% alcoholic solution of thymol-

phthalein and 30 ml of n-butyl alcohol. The solution is mixed and left to stand till it becomes clear. The clear solution is siphoned off into a bottle which is used to fill the absorbers.

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STUDY OF RATE OF WATER EXCHANGE IN FIELD CONDITIONS USING A BALANCE AND MOISTURE METER

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In the works of many investigators [1-11] it has been acknowledged that the study of the rate of utilization of soil water by field plants is becoming increasingly significant not only for theoretical research but also for the solution of a number of problems of agricultural practice.

In the field conditions, however, the direct determination of the water balance in plants is impossible and it is necessary to resort to indirect methods (Alekseev [1]). One such method is the study in field conditions of the variation of the ratio of transpiration to water content (water exchange) and the simultaneous measurement of the soil moisture. An effective means of rapidly determining the water content of weighed batches of vegetation and soil samples is an instrument (the universal field moisture meter) designed by P. P. Teterin (Fig. 1) which, as tests have shown (Table 1 and 2), fully meets the requirements of field work.

The measurement of the water content by the moisture meter is based on the principle of the condensation of the water contained in a weighed sample, a process which can be carried out in 7 minutes with this instrument.

The quantitative determination of the condensed water in the instrument is made by means of a float chamber, mirror and scale (Fig. 2). The method of mirror and scale permits a highly accurate determination of the angle of rotation of the mirror, which records the small weights of condensed water entering the float chamber.

The essence of the mirror and scale method is the following: given that the plane of the mirror (Fig. 3) is perpendicular to the plane of the drawing, the mirror E rotates around a horizontal axis. The beam of light from the source is directed on to the plane of the mirror, and after reflection falls on the scale.

In these conditions we will see on the scale the image of the source S at the point n_1. If the mirror turns so that its new position makes an angle α with its initial position, then the image of the source S will coincide with the scale division n_2. The absolute difference between the readings $n_2 - n_1$ enables us to determine the angle of rotation of the mirror, which is proportional to the weight of condensed water which has entered the float chamber. This has been confirmed by a large number of tests carried out by us.

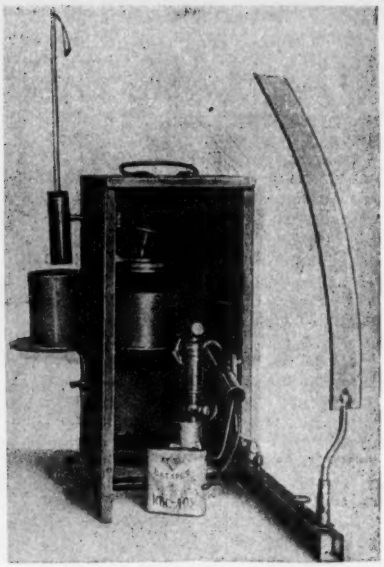

Fig. 1. General view of the universal field moisture meter.

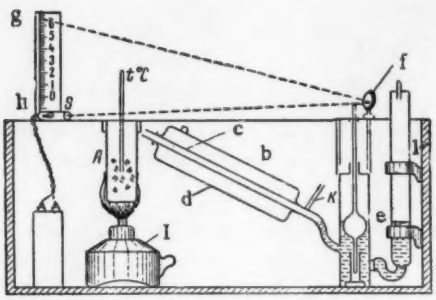

Fig. 2. Scheme of universal field moisture meter (explanation in text).

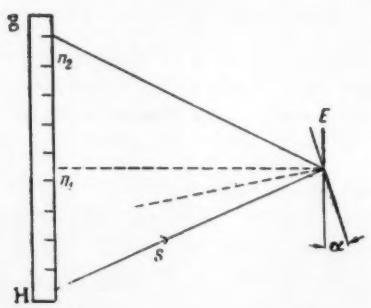

Fig. 3. Diagram of mirror and scale of universal field moisture meter (explanation in text).

The suggested method permits a reasonably accurate measurement of the water content (Tables 1 and 2). The instrument itself (Fig. 2) consists of the following parts:

1) heater A, internal diameter 20 mm, length 67 mm; 2) condenser B, consisting of two cylinders: an inner C with diameter 10 mm, length 150 mm, made of pure copper and an outer D, diameter 74 mm, length 84 mm, made of iron; 3) a float chamber E, diameter 35 mm, length 77 mm; 4) mirror F mounted on a horizontal spindle carrying a crank; 5) light source (three-volt bulb supplied from a pocket torch battery); 6) divided scale GH; 7) mercury thermometer t reading up to 250°C; 8) spirit lamp I.

The upper opening of the heater is hermetically sealed with a rubber stopper carrying a thermometer, the bulk of which is buried at the center of the soil sample placed in the heater. The side opening of the heater is joined hermetically by a cone-piece to the inner cylinder of the condenser. The other end of the cylinder is soldered to the base of the float chamber.

When all parts of the apparatus (excluding the heater) have been made airtight the whole system of the moisture meter (condenser and float chamber) is filled with water. The float chamber is filled with water till the image of the light source coincides with the zero reading of the scale. A very accurate superposition of the image of the light source on the zero reading is achieved by means of a communicating vessel L, which can be moved up and down. Having set the image of the light source at the scale zero, 10 g of soil, the water content of which is to be determined, is placed in the heater and the opening of the heater is hermetically sealed with the rubber stopper carrying the thermometer. Then the heater is connected up by the cone-piece to the condenser of the soil or plant sample heated to 105°. When a temperature of 105° is reached, the heating is stopped and the scale reading taken. The number of divisions moved by the image of the light source on the scale gives a reasonably accurate measurement of the water content of the sample. The adsorbed gases which were present in the soil escape through the tube K.

The calibration of the scale of the moisture meter is carried out in the following manner.

Having fixed the initial position of the image of the light source on the scale, called the initial reading, we put 3 g of water in the float chamber and note the position of the image of the light source on the scale. Then, by means of the vessel L we bring the image of the light source on the scale back to the initial reading, put 2 g of water in the float chamber and again note the position of the image of the light source on the scale.

As an example we give on Table 1 the results of one set of tests for calibration of the scale.

TABLE 1

Calibration of Scale of Field Moisture Meter

No.	Amount of water in g	Position of image of light source on scale (in scale divisions)		No.	Amount of water in g	Position of image of light source of scale (in scale divisions)	
		initial	final			initial	final
1	3.00	0	30.30	6	2.50	0	25.30
2	2.00	0	20.00	7	1.60	0	16.50
3	1.00	0	10.10	8	1.80	0	18.00
4	0.50	0	5.00	9	2.30	0	23.40
5	1.50	0	15.40	10	0.80	0	8.20

TABLE 2

Measurement of Water Content by Soil by Drying in Thermostat and by Condensation Method with the Universal Moisture Meter

Depth at which soil samples for analysis were taken in cm	Drying in thermostat		Condensation method (Universal moisture meter)	
	weight of soil after expt. in g	water content in %	weight of soil after expt. in g	water content in %
10	43.360	13.28	8.600	14.00
20	43.480	13.04	8.650	13.50
30	42.150	15.70	8.460	15.40
40	42.350	15.30	9.520	14.80
50	43.300	15.40	8.600	14.00
60	42.900	14.20	8.540	14.60
70	43.910	12.18	8.780	12.20

An examination of the data on Table 1 shows that the readings of the position of the image of the light source on the scale are directly dependent on the amount of water added to the float chamber.

If the position of the image of the light source on the scale is known, then the weight of condensed water can be found from Table 1. The accuracy of measuring the amount of condensed water using the described apparatus (with distance of scale from mirror 25 cm) is 0.01 g. Having determined from the scale the weight of condensed water, we calculate the water content of the specimen (percent water content in weighed sample) in percent from the formula: $x = \frac{100 \cdot p}{M}$, where M – mass of weighed sample: p – mass of condensed water.

We compared the determinations of the water content by the described field method with the usual laboratory method of drying.

In a field we cut a 1 meter-deep trench with vertical sides. The soil samples were taken along the trench at depths 10, 20, 30, 40, 50, 60 and 70 cm below the soil surface. Two samples of soil were taken at each depth. Dishes containing the soil samples were dried in a thermostat at 105°. At the same time the water content of these samples was measured by the field moisture meter. Data are given in Table 2.

Table 2 gives the results of measuring the water content of soil (foothill chernozem) in one set of experiments.

We see from Table 2 that the differences in water content, obtained with the usual laboratory drying method and the field method we have described, vary between ±0.2% and 0.68%.

Thus, Table 2 shows that it is possible, by using the instrument of P. P. Teterin, to determine within a few minutes in field conditions and with an accuracy of no less than that of the laboratory drying method, the water content in any plant organs or soil samples during physiological field experiments.

Using the apparatus of P. P. Teterin and a torsion balance for determining transpiration (according to Ivanov [6]), we studied the variation of the rate of water exchange $\frac{\text{transpiration}}{\text{water content}}$ in a whole series of field plants. Samples of leaves were taken from the same story every 2 hours and the rate of transpiration was expressed in milligrams per 1 hour per 1 g of wet matter. Then in the same leaf samples in the field, using the Teterin moisture meter (Figs. 2 and 3) we determined the water content and converted it to mg per 1 g of wet weight. Carrying out the calculations and determining the ratio: $\frac{\text{transpiration in mg}}{\text{water content in mg}}$ we found the 'coefficient of water exchange,' i.e., the rate of replacement of water in the plant leaves. For example, on April 9, 1957 at 9 a.m. in the tussock grass (Festuca sulcata Hack.) on an old bed, the water content of the leaves measured by the Teterin moisture meter was 561 mg per 1 g of wet weight, and the rate of transpiration 2979 mg per 1 g of wet weight. The coefficient of water eschange $\frac{\text{transpiration 2979 mg}}{\text{water content 561 mg}} = 5.3$, i.e., in tussock grass in field conditions the water was replaced more than five times every hour.

By means of a torsion balance and the Teterin moisture meter it is possible for a single worker in the field to study simultaneously the daily behavior of the rate of water exchange in several species of plants, and still be able to make rapid determinations of the change in water content of the surface horizons of the soil.

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ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci.-Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED

[Biological Sciences]

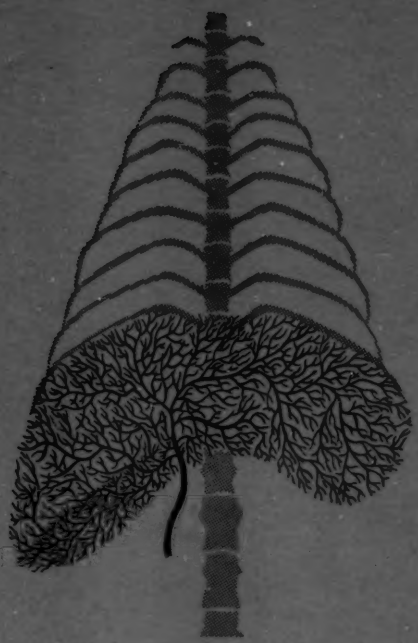
Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiia	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiia	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Embriol.	Arkhiv Anatomii Gistologii i Embriologii	Archives of Anatomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiia	Biokhimiia	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiia Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Biull. Eksptl. Biol. i Med.	Biulleten Eksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Biull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Biulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Eksptl. Khirurg.	Eksperimentalnaia Khirurgiia	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiia i Toksikologiia	Pharmacology and Toxicology
Farmatsiia	Farmatsiia	Pharmacy
Fiziol. Rastenii	Fiziologiia Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenova Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariia	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiia Akademii Nauk SSSR, Seriia Biologicheskaia	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiia Tikhookeanskogo N. I. Instituta Rybnogo Khoziaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiia	Khirurgiia	Surgery
Klin. Med.	Klinicheskaia Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo(po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaia Parazitologiia i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaia Radiologiia	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiia	Mikrobiologiia	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhiat. i Psikhogig.	Nevropatologiia, Psikhiatriia i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediia, Travmatologiia i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriia	Pediatriia	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Endokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniia Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaia Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journals
Stomatologiia	Stomatologiia	Stomatology
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratorii	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics

* LIBRARY OF CONGRESS TRANSLITERATION SYSTEM.

(continued)

(continued)

Abbreviation	Journal	Translation
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiia	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiia	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiia, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoiuz. Gidrobiol. Obshchestva	Trudy Vsesoiuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoiuz. Inst. Eksptl. Med.	Trudy Vsesoiuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiia	Urologiia	Urology
Uspekhi Biokhimiia	Uspekhi Biokhimiia	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiia	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriia Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universiteta, Seriia Biologii i Pochvovedeniia	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiia	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariia	Veterinariia	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniia	Voprosy Pitaniia	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaia Laboratoriia	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiia	Journal of General Biology
Zhur. Vysshei Nerv. Deiatel.	Zhurnal Vysshei Nervnoi Deiatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology



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Problems in the Classification of Antagonists of Actinomycetes. By G. F. Gause. Edited by David Gottlieb. Translated by Fritz Danga. Ready early 1959. $5.00 (U.S. & Canada) $5.50 (Foreign)

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